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# CHROMOSOMES AND PHYLOGENY IN CREPIS

BY

LILLIAN HOLLINGSHEAD and ERNEST B. BABCOCK

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## INTRODUCTION

In connection with genetic and taxonomic studies of *Crepis*, an examination of as many species as could be brought into cultivation has been in progress for about ten years. The earlier work on the chromosomes was done by Dr. Margaret Mann Lesley, who studied particularly numbers and sizes (Mann, 1922, 1925; Babcock and Lesley, 1926). The work of M. Navashin (1925, 1926) and Taylor (1925, 1926), who described satellites and constrictions for the first time in this genus, showed that a closer morphological study of the chromosomes from suitably fixed material would be of value for comparative studies of related species.

It is the purpose of this paper to present our knowledge of number and morphology of the chromosomes in seventy species and to consider this evidence in relation to a system of classification based on phylogenetic relationship. But the present paper is not intended to serve as a taxonomic treatise. Therefore no keys or descriptions of species will appear and there will be no attempt to set forth the detailed evidence for the phylogenetic groupings proposed, as such descriptions and data will appear in a taxonomic treatment now in preparation. The specific names used have been carefully verified as to identity, priority, and authorship, and are in nearly every case the same as those which will be used in later publications. In the present paper it is proposed merely to present the evidence derived from cytological investigation and to discuss the phylogenetic groupings of those species of *Crepis* which have been investigated. These groupings, however, have been worked out by combining the data on chromosome number and morphology with the evidence from external morphology of the plants, at the same time giving consideration to geographic distribution and to the genetic evidence derived from experiments on interspecific hybridization.

The point of view held by the writers with regard to the fundamental relations between phylogeny and taxonomy is in general

agreement with that of Hall and Clements (1923). The concept of species defined by these authors and accepted by us as a satisfactory approximation to the truth is stated as follows:

The evolutionary view of the species is that it is a definite phylogenetic stock, sprung from and related to similar stocks, and itself undergoing modification into a number of variads. As they have recently come from the same stock, these variads are more nearly related to each other than they are to those of any other species, and they represent a definite phylogenetic unit, the species, at the same time that they mark its further differentiation.

But the view of these authors, that gross morphological difference between plants, as contrasted with such cytological features as number and morphology of the chromosomes, is the only definite measure of progress in evolution, is too limited, as has already been pointed out by one of us (Babcock, 1924). The importance of the chromosomes, especially their appearance in somatic cells at mitotic metaphase, as an index of taxonomic relationship, has become increasingly evident as the number of species of *Crepis* examined has increased.

#### ACKNOWLEDGMENTS

It is impossible at this time to mention the many institutions and individuals who have assisted by providing seeds or roots of the species herein discussed. These will appear in connection with sources to be acknowledged later. We are especially indebted, however, to Dr. M. Navashin for seeds of several species, which he brought from Russia, and for active interest and frequent help throughout the investigation. We also gratefully acknowledge the constant cooperation of Mr. C. W. Haney in connection with growing the plants, assisting in collection of root tips, and many other details.

#### CYTOLOGICAL MATERIALS AND METHODS

Somatic metaphases of root tips have been used exclusively for chromosome counts and morphological studies. In most cases the material was obtained from plants in the rosette stage after they had been transplanted to five- or six-inch pots. Occasionally the roots of very young seedlings were used but they were on the whole less satisfactory. Some of the counts on the American species were procured from tap roots dug from the wild and transplanted to pots in the greenhouse, where they produced new roots, giving material well suited for chromosome studies. In other American species the counts

were obtained from young seedlings. Introduction of most American species into garden culture has been found very difficult but is gradually being accomplished.

Roots were fixed in Taylor's (1925a) chrom-acetic-osmic-maltose solution, in weak and strong Flemming, and in chrom-acetic-formalin. Most of the investigations and all of the drawings have been made from material fixed in one of the two chrom-acetic-formalin solutions given below.

- |  |  |
|--|--|
| 1. A. 65 cc. water                                 | 2. A. 65 cc. water                                 |
| 10 cc. glacial acetic acid                         | 10 cc. glacial acetic acid                         |
| 1 gr. chromic acid                                 | 1 gr. chromic acid                                 |
| B. 40 cc. formalin (commercial)                    | B. 10 cc. formalin (commercial)                    |
| 35 cc. water                                       | 65 cc. water                                       |
| Mix one part A with one part B just before fixing. | Mix one part A with one part B just before fixing. |

No constant difference in quality of fixation between material fixed in these two solutions has been established. The chromosomes fixed with these fixatives are usually slightly shorter and broader than those fixed in the osmic fixatives, so they may be somewhat contracted. Morphological details are quite clear in well fixed material, however, and in material so fixed the homogeneity of the cytoplasm, against which the chromosomes stand out very clearly, facilitates greatly the examination of the sections for suitable figures.

The material was imbedded in paraffin and sections were cut 6 to 12 $\mu$  thick depending on the size and number of the chromosomes. Heidenhain's iron haematoxylin was used for staining.

A Bausch and Lomb (90 $\times$ ) apochromatic oil immersion-objective and Zeiss compensating oculars have been used in this study. All the drawings have been made with the help of a camera lucida at a magnification of approximately 3750 and reduced to 2500 in reproduction.

Where the chromosomes of a species were being investigated for the first time, counts from two or more plants were made whenever possible. The number of plants investigated is indicated in the table of chromosome numbers. In those instances where the chromosome number of a species newly investigated is based on one plant this plant is the only one which has been grown in our cultures. In the case of *Crepis incana*, the plant whose chromosomes were studied did not bloom; and as this was the only plant available and there were no herbarium specimens accompanying the seeds, the identification was based on achene and leaf characters alone.



Where the species had 12 or fewer chromosomes an attempt was made to find a metaphase plate, a drawing of which would depict the complex so that each individual chromosome might be recognized if it could be distinguished from the others under the microscope. Lack of sufficient material or particular difficulties have in some cases forced us to be satisfied with figures of plates which do not reach this ideal. In most cases, however, the chromosomes can be distinguished in the figures and the pairs recognized without difficulty.

Where the number exceeds 12, the problem of distinguishing the various chromosomes becomes more difficult and in most cases it has been impossible to distinguish all the chromosome pairs in one plate, although various types are readily picked out. In those species with more than twenty chromosomes the plates for drawing were chosen with the primary aim of depicting clearly the number, and, secondarily, of showing as many as possible of the chromosomes which could be distinguished by their particular morphology.

## GENERAL MORPHOLOGICAL FEATURES

Marked differences in the frequency of occurrence of good metaphase plates were noted between species. The particular species in question, as well as the condition of the roots, affected the number of mitotic figures. Some species characteristically showed plates in which the chromosomes were well spaced in a single plane while in others the usual arrangement of chromosomes was such that a morphological study was impossible. The species with higher numbers and larger chromosomes presented greater difficulties in this respect.

Most species, in material well fixed in chrom-acetic-formalin and well stained, show black chromosomes, their outlines clear and distinct against the gray cytoplasm. When differentiated so as to clear the cytoplasm, some species show chromosome outlines which are less sharp, especially under high magnification. The extreme phase of this condition was found in certain species (*C. bulbosa* and *C. japonica*) which have been included under *Crepis* but which at one time or another have been assigned to other genera. The same is true of *Ixeris graminea*, which for a time was thought to belong in *Crepis*. Their chromosomes are small, and it may be that this is the reason why the outlines under differentiation become less clear so as to make it difficult to determine the shapes with exactness. The black and white figures used in this paper are not suitable for showing such differences.

Size difference continues to be one of the most useful features by which chromosomes may be distinguished. The relative lengths of the components of a particular chromosome complex are rather constant and although the lengths of the same chromosome may differ noticeably from cell to cell this variation is relatively small. Differences in the thickness of chromosomes of different species are obvious but no consistent difference between the widths of the various chromosomes of any one complex has been observed.

Satellites or "trabanten," chromatin balls attached to the chromosomes proper by thin threads, first described by S. Navashin (1912), are now generally recognized as of wide-spread occurrence. The list of plants and animals in which satellites have been observed, given by Kuhn (1928), will serve to illustrate in what diverse groups these peculiar structures occur. Most, if not all, species of *Crepis* contain at least one pair of chromosomes marked by the presence of satellites. They vary in size from a large ball (*C. setosa*, fig. 4c) to a tiny sphere (*C. palaestina*, fig. 18d). As in most other genera they are usually attached to the proximal ends (those which lie toward the center of the plate) of chromosomes which have short arms or heads (cf. below), but in some cases (*C. biennis*, fig. 12a) they may be attached to chromosomes with arms more nearly equal in length. In only one species (*C. pulchra* fig. 19) was there found a pair of chromosomes with distal satellites. In several species (*C. lyrata*, *C. mollis*, *C. hierosolymitana*, fig. 13) structures resembling large satellites at times, but at others appearing to be segments cut off by distal constrictions, have been seen. Such variations may be due to imperfect fixation as suggested by Taylor (1924). In this connection it should be noted that Geitler (1929) figured the chromosome complexes of *C. capillaris*, *C. dioscoridis*, *C. rubra*, and *C. blattarioides*, depicting morphological features similar to those shown in our figures. Considerable variation in the appearance of the satellited chromosome of *C. blattarioides* was described, including the form and size of the satellite and even its presence or absence. Some if not all of this variation can perhaps be attributed to poor fixation.

The behavior of the satellites during the mitotic anaphase of *C. capillaris* has been described and figured by Taylor (1926) and the writers' observations add nothing new to this knowledge. Whether or not the satellites are to be found on the nucleolus at prophase as stated by M. Navashin (1925) has not been particularly studied but prophases have been seen which could be interpreted as exhibiting this phenomenon.

The clearness with which the satellites are visible depends on their size, the quality of the fixation, and the degree of differentiation. The thread which attaches the satellite to the chromosome proper may be contracted under poor fixation until a small satellite appears as a little protuberance (*C. incarnata*, fig. 18c) or a large satellite as a segment. Excessive or even ordinary differentiation may render invisible a satellite thread or a small satellite. They are not always to be seen even in well fixed material but it is believed that in such cases they are hidden by the chromosomes proper and that they are constant morphological features of the living chromosome. The satellite may vary slightly in apparent size and shape from cell to cell within a species but on the whole it is as constant as any morphological feature. On occasion a satellite thread may be drawn out to an unusual length (*C. amplexifolia*, fig. 5c) or the spherical shape may be obliterated (*C. parviflora*, fig. 11a). These, however, are exceptional cases and may be due to poor fixation.

One certain case (*C. foetida* 2048, fig. 1d) and several possible cases of a constant difference in size between satellites on homologous chromosomes within a species have been found. M. Navashin (1926) reported such a case in *C. dioscoridis* and similar cases in other genera are known (cf. Kuhn, 1928). Detailed studies of more material would be necessary to decide the questionable cases.

Aside from size and the occurrence of satellites, a number of investigators have found that the most useful distinguishing feature of chromosomes is the occurrence of a fiber attachment constriction dividing the chromosome into two arms. The importance of the "two-armed" nature of the chromosomes has been reemphasized by Heitz (1928), who has found it in many widely separated families and indeed in different divisions of the plant kingdom, and he holds it to be a universal characteristic of chromosomes. S. Navashin (1916b) has stated that absolutely every chromosome possesses an "achromatic transverse fissure" which divides the chromosome into two arms (cited from M. Navashin, 1926). Taylor (1925, 1926), who has discussed fully the kinds of constrictions, has failed to find a case among any of the medium or large chromosome types studied which had a truly terminal fiber attachment and he is inclined to the opinion that an actual constriction zone or related structural differentiation is always present and can be demonstrated by suitable means. All investigators agree that one arm may be so short as to constitute a small head.

In *Crepis*, fiber attachment constrictions have been found on practically every chromosome which could be identified (except in *C. japonica* and *C. bulbosa*). Where the chromosome is bent so as to obscure the constriction, the relative lengths of the arms give a fair indication of its position, for the chromosome usually bends at the constricted region. The constrictions are particularly apt to be observed on chromosomes where they are to be found very near the ends, as on the satellited chromosomes of *C. alpina* var. *syriaca* Bornm.<sup>1</sup> (fig. 2c).

### THE CHROMOSOMES OF SEVENTY SPECIES

Mann (1925) summarized the chromosome numbers of the twenty-seven species of *Crepis* reported by various investigators up to that time and Tischler (1927) has a complete bibliography of papers dealing with *Crepis* chromosomes which includes additional counts given by M. Navashin (1925), Babcock and Lesley (1926), and some unpublished chromosome counts by M. Navashin. The chromosome number of *C. reuteriana* has been given by Babcock and Hollingshead (1929). In no case have counts obtained by the writers differed from those reported by Mann, Navashin, or Babcock and Lesley.

With a few minor exceptions, no details of somatic chromosome morphology other than size differences were described in earlier works on the genus. De Smet (1913-14) figured satellites on *C. virens* (= *C. capillaris*) but did not describe them. Rosenberg (1918) recognized flexures in *C. tectorum* chromosomes as constant in position at somatic anaphase and later (1920) says, speaking of the chromosomes of *C. capillaris* (wrongly called *C. reuteriana*, cf. Babcock, 1924b), "ein sog. Trabantenbildung kommt vor die aber meiner ausicht nach von ganz anderer Natur ist als die 'Quersegmentierung.'" It is likely that the so-called "trabanten" which he saw were merely heads separated from the chromosomes proper by constrictions. De Litar-di re (1923) located the point of fiber attachment on one chromosome of *C. virens* (= *C. capillaris*) and noted that the chromosome was usually bent at that point. Mann (1925) saw the large satellite of *C. setosa* and stated later (Babcock and Lesley, 1926) that satellites were "not always present" in her material. Such results can, in most cases, be attributed to the use of unsuitable fixatives.

<sup>1</sup> This "variety" should be recognized as a distinct species, but the change in nomenclature will not be made at this time.

M. Navashin (1925, 1926) and Taylor (1925*b*, 1926) established the constant occurrence of satellites and fiber-attachment constrictions as features of use in distinguishing the various chromosomes in a number of species of the genus. Navashin (1925) figured ten species in which he had determined the morphological features of each chromosome. These species were *C. pulcherrima* (= *C. pulchra*), *C. grandiflora* (= *C. conyzaefolia*), *C. dioscoridis*, *C. tectorum*, *C. rubra*, *C. marschalli*, *C. virens* (= *C. capillaris*), *C. rhoeadifolia* (= *C. foetida*), *C. alpina*, and *C. parviflora*. To these he has added *C. aspera* in another paper (1927). Taylor (1925*b*) gave figures showing the morphological features of the various chromosomes of *C. setosa* and *C. capillaris*, and later (1926) he figured *C. capillaris* in somatic metaphases and anaphases. The investigations reported here have corroborated those of Navashin and Taylor in practically every detail, and drawings of the species they figured are included only for the sake of uniformity and completeness.

The present investigations add twenty-seven species to the list of those whose chromosome numbers are known (including four species of other genera closely related to *Crepis*). Table 1 gives the somatic

TABLE 1

## THE SOMATIC CHROMOSOME NUMBERS OF SIXTY-SEVEN SPECIES OF CREPIS

(Three other species are listed, with numbers in italics, which are not accepted in *Crepis* by the authors.)

## OLD WORLD SPECIES

| Species                                       | Somatic chromosome number | Number of plants examined | Accession        |
|---|---------------------------|---------------------------|------------------|
| <i>C. aculeata</i> (DC.) Boiss.               | 8                         | 2                         | 1602             |
| <i>C. alpina</i> L. . . . .                   | 10                        | 1                         | 1499             |
| * <i>C. alpina</i> var. <i>syriaca</i> Bornm. | 10, 11, 12, 13            | 20                        | 1923             |
| <i>C. amplexifolia</i> (Godr.) Willk.         | 8                         | 5                         | 1019             |
| <i>C. aspera</i> L. . . . .                   | 8                         | 2                         | 1135, 1973       |
| * <i>C. asturica</i> Lacaita . . . . .        | 10                        | 3                         | 2088             |
| <i>C. aurea</i> (L.) Cass. . . . .            | 10                        | 2                         | 2170             |
| <i>C. biennis</i> L. . . . .                  | 39, 41                    | 5                         | 1874, and others |
| <i>C. blattarioides</i> (L.) Vill. . . . .    | 8                         | 1                         | 2033             |
| <i>C. bulbosa</i> (L.) Tausch . . . . .       | 18                        | 3                         | 1303             |
| * <i>C. bungei</i> Ledeb. . . . .             | 8                         | 16†                       | 1827             |
|   | 16                        | 3                         | 2174             |
| * <i>C. burejensis</i> F. Schmidt . . . . .   | 8                         | 5                         | 2747             |
| <i>C. bureniana</i> Boiss. . . . .            | 8                         | 8                         | 1655             |

\* Chromosome number reported for the first time.

† One plant was triploid with 12 chromosomes.

TABLE 1—(Continued)

| Species                                       | Somatic chromosome number | Number of plants examined | Accession         |
|---|---------------------------|---------------------------|-------------------|
| <i>C. bursifolia</i> L.....                   | 8                         | 3**                       | 1220              |
| <i>C. capillaris</i> (L.) Wallr. . . . .      | 6                         | 20                        | X, 982 and others |
| <i>C. chondrilloides</i> Jacq. . . . .        | 8                         | 2                         | 2180, 1907        |
| * <i>C. chrysanthia</i> Froel. . . . .        | 8                         | 3                         | 2179              |
| <i>C. ciliatq</i> C. Koch . . . . .           | 40, 42?                   | 2                         | 2181              |
| <i>C. conyzaefolia</i> (Gouan) D. T.          | 8                         | 2                         | 2183              |
| <i>C. dioscoridis</i> L. . . . .              | 8                         | 3                         | 1742, 972, 1455   |
|   |                           | 3                         | 1751, 2188        |
| <i>C. foetida</i> L... . . . .                | 10                        | 1                         | 2048              |
|   |                           | 3                         | 2307              |
| * <i>C. gymnopus</i> Koidz... . . . .         | 8                         | 2                         | 2746              |
| * <i>C. hackeli</i> Lange . . . . .           | 16                        | 3                         | 1873              |
| * <i>C. hierosolymitana</i> Boiss. . . . .    | 12                        | 3                         | 2619              |
| <i>C. hookeriana</i> Ball . . . . .           | 8                         | 1                         | 1458              |
| * <i>C. incana</i> Sibth. et Sm. . . . .      | 16                        | 1                         | 1667              |
| <i>C. incarnata</i> Tausch . . . . .          | 8                         | 1                         | 1304              |
| <i>C. japonica</i> (L.) Benth. . . . .        | 16                        | 2                         | 1045, 2132        |
| <i>C. lacera</i> Tenore . . . . .             | 8                         | 4                         | 1914              |
| * <i>C. leontodontoides</i> All. . . . .      | 10                        | 1                         | 1807              |
| * <i>C. lybica</i> Pamp. . . . .              | 8                         | 1                         | 1698              |
| <i>C. lyrata</i> Froel. . . . .               | 12                        | 4                         | 1644              |
| <i>C. marschalli</i> C. A. Mey. . . . .       | 8                         | 3                         | 1532              |
| <i>C. mollis</i> (Jacq.) Asch. . . . .        | 12                        | 2                         | 2201              |
| <i>C. montana</i> Urv. . . . .                | 12                        | 2                         | 1175              |
| <i>C. multicaulis</i> Ledeb. . . . .          | 10                        | 2                         | 1480              |
| <i>C. myriocephala</i> Coss. et DR. . . . .   | 8                         | 1                         | 1557              |
| * <i>C. nana</i> Richards. . . . .            | 14                        | 2                         | 2698              |
| <i>C. neglecta</i> L. . . . .                 | 8                         | 1                         | 1753              |
| <i>C. nicaeensis</i> Balb. . . . .            | 8                         | 6                         | 2700              |
| <i>C. palaestina</i> (Boiss.) Bornm. . . . .  | 8                         | 1                         | 1552              |
| <i>C. paludosa</i> (L.) Moench . . . . .      | 12                        | 1                         | 1825              |
| <i>C. pannonica</i> (Jacq.) C. Koch . . . . . | 8                         | 1                         | 1695              |
| <i>C. parviflora</i> Desf. . . . .            | 8                         | 2                         | 1630              |
| * <i>C. pontana</i> (L.) D. T. . . . .        | 10                        | 2                         | 2204              |
| <i>C. praemorsa</i> (L.) Tausch . . . . .     | 8                         | 3                         | 2133              |
|   |                           | 4                         | 1213, 1483        |
| <i>C. pulchra</i> (L.) . . . . .              | 8                         | 1                         | 1894              |
| <i>C. reuteriana</i> Boiss. . . . .           | 8                         | 4                         | 2134 2218         |
|   |                           | 2                         | 1506              |
| <i>C. rubra</i> L. . . . .                    | 10                        | 1                         | 1176              |
| <i>C. senecioides</i> Delile . . . . .        | 8                         | 5                         | 1044              |
|   |                           | 1                         | 1036              |
| <i>C. setosa</i> Hall. f. . . . .             | 8                         | 1                         | 1510              |
|   |                           | 2                         | 1862              |
| <i>C. sibirica</i> L. . . . .                 | 10                        | 5                         | 1806, 1064, 1704  |
| <i>C. taraxacifolia</i> Thuill. . . . .       | 8                         | 1                         | 1895              |

\* Chromosome number reported for the first time.

\*\* One plant was a trisomic with 9 chromosomes.

TABLE 1—(Continued)

| Species                                      | Somatic chromosome number | Number of plants examined | Accession          |
|--|---------------------------|---------------------------|--------------------|
| <i>C. tectorum</i> L.....                    | 8                         | 9                         | 1498               |
|  |                           | 1                         | 1702               |
| * <i>C. tenuifolia</i> Willd.....            | 15                        | 8                         | 1826               |
| <i>C. tingitana</i> Salz.....                | 10                        | 1                         | 1681               |
| <i>C. vesicaria</i> L.....                   | 8                         | 4                         | 1576               |
| * <i>Rodigia commutata</i> Spr.....          | 10                        | 2                         | 1666, 2219         |
| * <i>Izeris graminea</i> Nakai.....          | 16                        | 2                         | 2568               |
| * <i>Pterotheca sancta</i> (L.) K. Koch..... | 10                        | 1                         | 2582 or 2583       |
| AMERICAN SPECIES                             |                           |                           |                    |
|  | 44?                       | 1                         | 1778 (typical)     |
|  | 33                        | 6                         | 1922 (typical)     |
|  | 33                        | 1                         | 2096 (typical)     |
| * <i>C. acuminata</i> Nutt.....              | 55?                       | 1 or more†                | 1830 (form)        |
|  | 33                        | 1 or more                 | 1848 (form)        |
|  | 33                        | 4                         | 1919 (form ?)      |
|  | 33                        | 4                         | 1934 (hybrid form) |
| * <i>C. andersoni</i> Gray                   | 22                        | 3                         | 2086 (typical)     |
|  | 22                        | 2                         | 2136 (form)        |
| * <i>C. elegans</i> Hook.....                | 14                        | 5                         | 2654               |
|  | 88?                       | 1 or more                 | 1840 (typical)     |
| * <i>C. barbiger</i> Leib.                   | 88?                       | 1 or more                 | 1842 (typical)     |
|  | 44                        | 1 or more                 | 1838 (hybrid form) |
|  | 88?                       | 6                         | 1959 (hybrid form) |
| * <i>C. glauca</i> (Nutt.) T. and G.         | 22                        | 1 or more                 | 2079 (typical)     |
| * <i>C. gracilis</i> (Eat.) Rydb.            | 22                        | 2                         | 2572 (typical)     |
|  | 55?                       | 6                         | 1695 (form)        |
| * <i>C. monticola</i> Coville                | 55?                       | 1 or more                 | 2771 (typical)     |
| <i>C. nana</i> (see Old World Species)       |                           |                           |                    |
|  | 22                        | 2                         | 2772 (typical)     |
| * <i>C. occidentalis</i> Nutt.....           | 22?                       | 1                         | 2220 (form)        |
|  | 44                        | 5                         | 1921 (form)        |
|  | 22                        | 1                         | 1829 (form)        |
|  | 22                        | 1                         | 2075 (form)        |
|  | 22                        | 1                         | 2078 (form)        |
|  | 22                        | 1                         | 2066 (form)        |
|  | 22                        | 2                         | 2065 (form)        |
| * <i>C. runcinata</i> (James) T. and G.      | 22                        | 1                         | 2068 (form)        |
|  | 22                        | 1                         | 2069 (form)        |
|  | 22                        | 1 or more                 | 2076 (form)        |
|  | 22                        | 2                         | 2077 (form)        |
|  | 22                        | 1                         | 2083 (form)        |
|  | 22                        | 1                         | 2071 (form)        |
| * <i>C. scopulorum</i> Cov.....              | 44?                       | 2                         | 2773 (form)        |

\* Chromosome number reported for the first time.

† Indicates that the roots were fixed under the accession number only and no record was kept of the number of plants fixed together.

chromosome numbers of the species, the chromosomes of which are described below, the numbers of individual plants, and the accession numbers of the various plants examined. An accession number is given to each lot of seeds or roots when it is received and the progeny of any accession is likewise designated by that number. An asterisk denotes that the chromosome number is reported for the first time. Three of the species designated by asterisks (*C. lybica*, *C. runcinata*, and *C. glauca*) were first counted by Dr. Margaret Mann Lesley and one (*C. leontodontoides*) was first examined by Miss Priscilla Avery (unpublished observations).

With the discovery of the somatic number 14 in *C. nana*, the known series of somatic chromosome numbers in the Old World species of *Crepis* becomes 6, 8, 10, 12, 14, 15, 16, and  $40\pm$ . That of the American species is 14, 22, 33, 44, 55?, and 88?.

In the following descriptions the species are taken in groups corresponding to the phylogenetic groups to be considered later. There are four major groups or subgenera—**Paleya**, **Barkhausia**, **Catonia**, and **Eucrepis**. The arrangement of species within each of these divisions is shown in the chart on page 30.

### **Paleya**

*Crepis asturica* ( $2n=10$ ; fig. 1a), the one representative of the subgenus **Paleya** which has been examined cytologically, has a complex which illustrates several of the chromosome types common in the genus. The two longer pairs, nearly equal in length, can be distinguished from one another by a small difference in the relative lengths of the arms which are separated by the fiber attachment constrictions. The satellited pair, shorter, has the fiber attachment constrictions closely subterminal, forming small heads to which the small satellites are attached. The two remaining pairs, nearly equal in length to the satellited pair, have fiber attachment constrictions median or nearly median and they commonly appear as small V's.

### **Barkhausia**

The chromosomes of *C. alpina*, *C. alpina* var. *syriaca*, *C. rubra*, *C. foetida*, and *Rodigia commutata*, all with a somatic number of ten, resemble those of *C. asturica* but with the exception of *C. rubra* they are generally smaller. Those of *C. foetida* (fig. 1b, c, d) resemble those of *C. asturica* most closely and are almost as large.



*C. foetida* 2188 (= *C. rhoeadifolia*, Navashin, 1925) does not differ noticeably from typical *C. foetida* in chromosome morphology, nor does *C. foetida* 2307 (= *C. glandulosa* Guss.), but *C. foetida* 2048 (= *C. interrupta* S. et S.) is distinguished by larger satellites and the plant examined showed a marked difference in the size of the two satellites (fig. 1d). That a careful study involving investigation and measurement of many chromosomes might establish further differ-

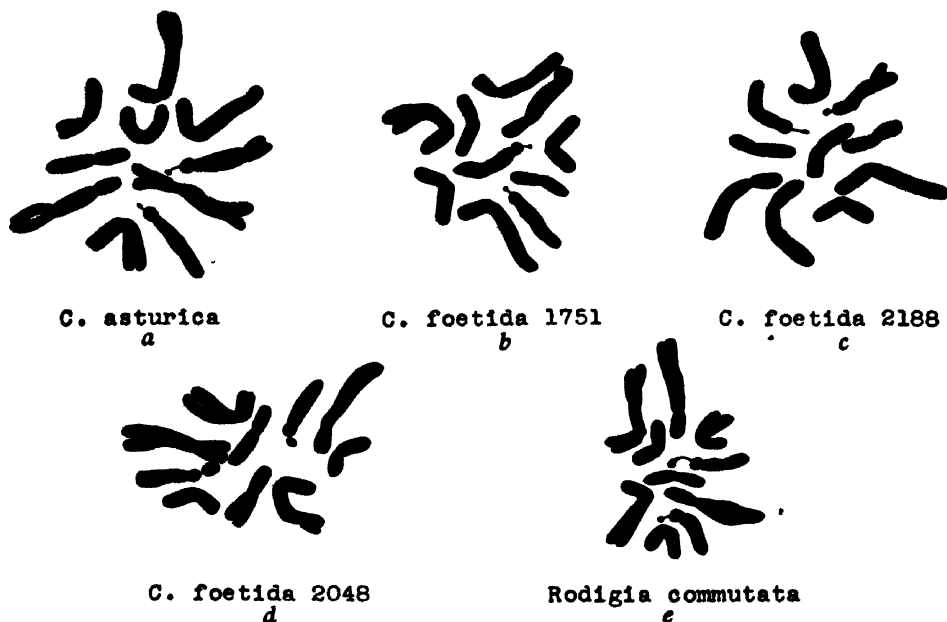


Figure 1.

ences between these *foetida* strains cannot be denied. *Rodigia commutata* (fig. 1e) has a chromosome complex very similar to that of *C. foetida*.

In *C. alpina* (fig. 2a) one of the two short pairs of chromosomes has small heads. Plants of *C. alpina* var. *syriaca* (fig. 2b, c, d, e) which were examined varied in chromosome number from 10 to 13. Table 2 gives the number of plants counted and their origins. With one exception only the first root of a seedling was examined and in many cases it was not possible to determine the shapes of all the chromosomes. However, it was established that at least some of the plants with 10 chromosomes had a chromosome garniture which differed noticeably from that of *alpina* in the shape of one of the small pairs. As far as could be determined the variation in number involved a small chromosome with a head to which was attached a rather large satellite, the 10-, 11-, 12- and 13-chromosome plants

TABLE 2  
THE SOMATIC CHROMOSOME NUMBERS FOUND IN *C. ALPINA* VAR. *SYRIACA*

| Origin of seed   | Culture number | Number of plants with chromosome numbers of |    |     |    |
|--|----------------|---|----|-----|----|
|  |                | 10  | 11 | 12  | 13 |
| Univ. Calif. Herbarium sheets 313831 and 313832* ..... | 27. 1923       |   | .. | 1   | .. |
| Univ. Calif. Herbarium sheet 313832 .....              | 28. 1923A      | 6   | 1  | ... | .. |
| 27.1923-2 open pollinated ..                           | 28 1923B       | 1   | 3  | 5   | 3  |

\*Several plants collected in Galilee, Palestine, environs of the Menahamiah Company, April 20, 1924, by M. Chijik.

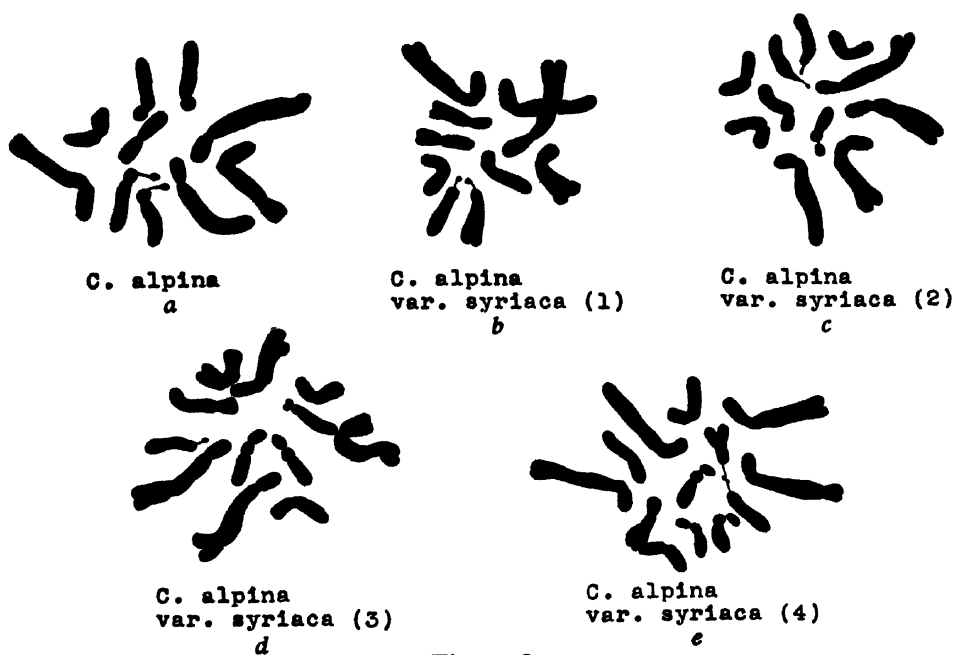


Figure 2.

having none, 1, 2, or 3 of these chromosomes respectively. This situation recalls that of the supernumerary chromosomes in maize (Randolph 1928a and b) and merits further study.

The chromosomes of *C. rubra* (fig. 3a, b) are larger than those of *C. asturica* and they differ in other respects, the most noticeable of which is the occurrence of a pair of short chromosomes with large, often constricted, satellites. No other satellites were seen in *C. rubra* 1506 but in *rubra* 1176 one chromosome pair bore very small satellites. M. Navashin (1925) shows the small satellites in his figure of *rubra* but in strains examined later he failed to find it (unpublished observations).

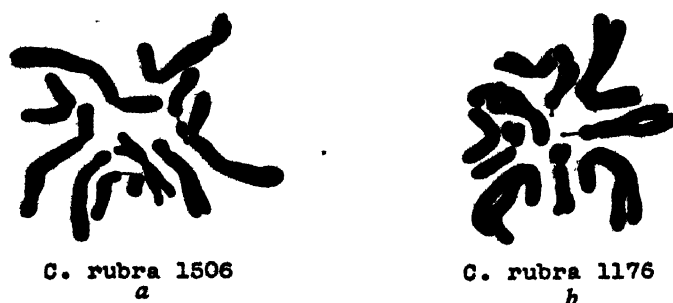


Figure 3.

*Crepis aspera*, *C. bursifolia*, *C. setosa*, and *C. senecioides* (fig. 4), with 8 chromosomes each, are quite different in details of their chromosome morphology. *C. setosa* is characterized by the presence of a pair of large satellites and *C. senecioides* is outstanding among the 8-chromosome species by the small size of its chromosomes.

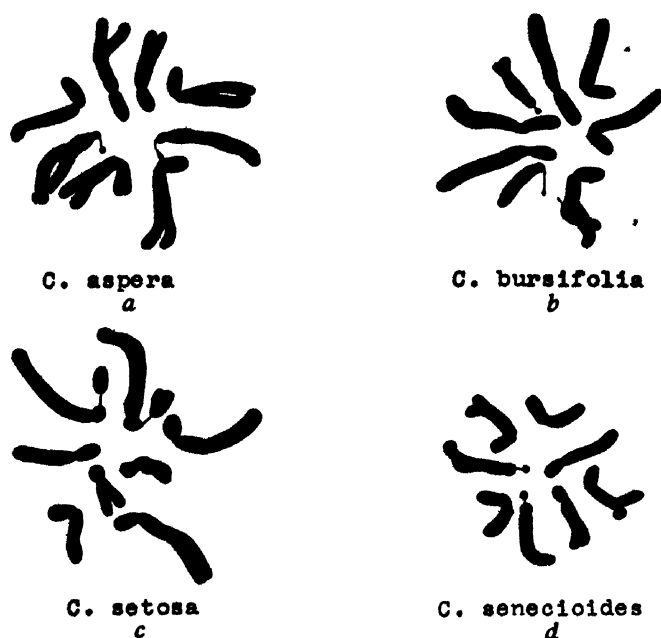


Figure 4.

*Crepis bureniana*, *C. aculeata*, and *C. amplexifolia* (fig. 5) with 8 chromosomes, are quite distinct in chromosome morphology. *C. bureniana* and *C. aculeata* have chromosomes of the same size order; those of *C. amplexifolia* are smaller. The exact position of the fiber attachment constriction on one chromosome of *bureniana* has not been determined, owing probably to inferior fixation, but it is very near the end.



Figure 5.

The chromosome garnitures of *C. myriocephala* ( $2n=8$ ) and *C. lybica* ( $2n=8$ , fig. 6a, b) appear to be indistinguishable from each other and differ only in their slightly larger size from those of *C. vesicaria*, *C. taraxacifolia*, and *C. marschalli* (fig. 6c, d, f) which are extremely similar to one another.

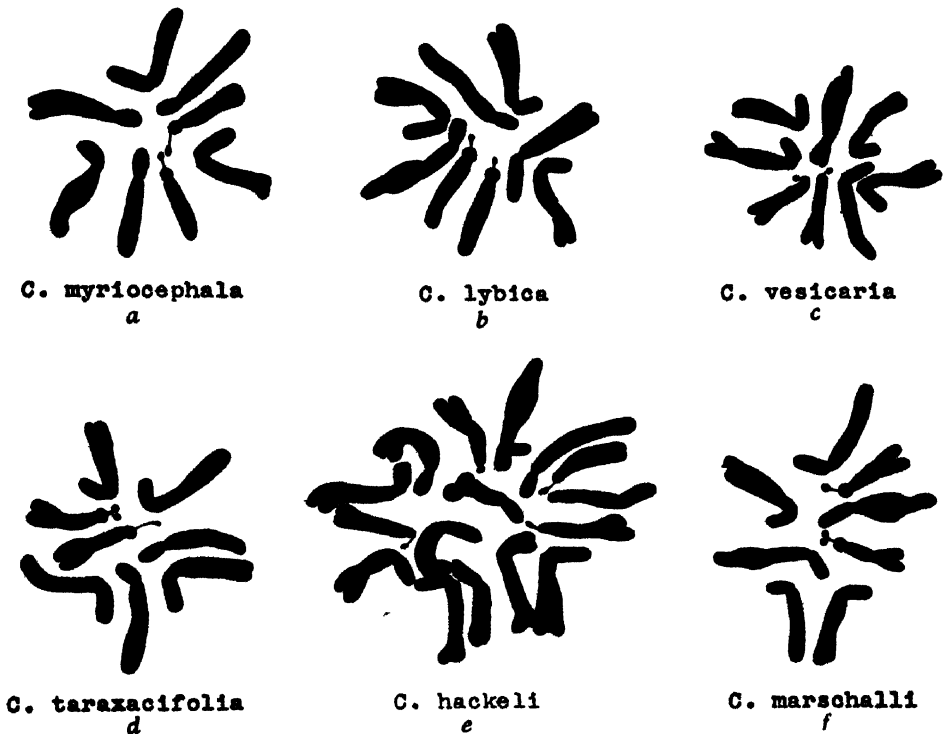


Figure 6.

*Crepis hackeli* ( $2n=16$ , fig. 6e) is probably a tetraploid species. Good plates showed 4 similar satellited chromosomes resembling closely those seen in *vesicaria*, etc., and 4 chromosomes resembling the

largest of those in the same group of species. The increased number did not permit an exact comparison of the remaining chromosomes which are rather similar in morphology. They do, however, resemble in shape and size the intermediate chromosomes of the *vesicaria* group.

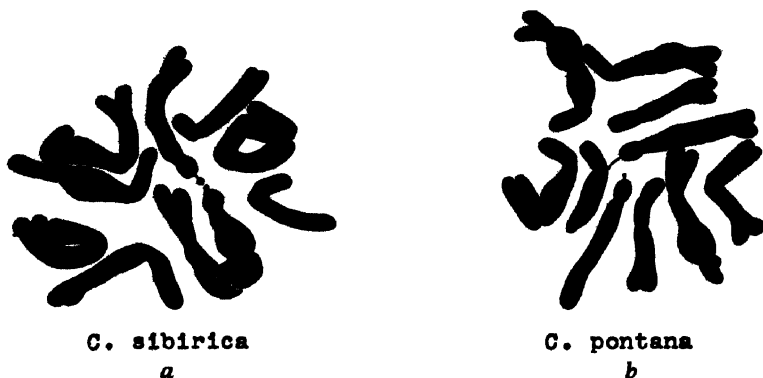


Figure 7.

### Catonia

*Crepis sibirica* and *C. pontana* ( $2n=10$ ) have chromosome complexes which differ in details but are similar in size and much larger than those of any other 10-chromosome species examined (fig. 7). In the plate of *C. sibirica* figured, one of the satellite threads has contracted so that the satellite appears as a small protuberance on the proximal end of the chromosome. This is not a constant condition.

*Crepis blattarioides* ( $2n=8$ , fig. 8a) has, on the whole, chromosomes slightly smaller than those of other species of this phylogenetic line. One pair bears rather large satellites. *C. burejensis*, *C. chrysanthia*, and *C. conyzaefolia* (fig. 8b, c, d) have similar garnitures of 8 large chromosomes, the first differing noticeably from the other two in the shape and size of the satellited chromosome. In the plate from which the figure of *C. chrysanthia* was drawn, one of the satellites, two of which were found in other plates, was apparently hidden by another chromosome.

*Crepis paludosa* ( $2n=12$ ) is easily distinguished from the other species of this subgenus which have been examined cytologically by the number and shape of its chromosomes (fig. 8e).

In *C. aurea* ( $2n=10$ , fig. 9a) the chromosome complex resembles that of *C. asturica*, differing markedly from it in only one chromosome pair. The chromosomes of *C. bungei* 1827 ( $2n=8$ , fig. 9c) are larger than those of *C. hookeriana* ( $2n=8$ , fig. 9b). *C. bungei* 2174 ( $2n=16$ , fig. 9d) has chromosome types resembling those of *C.*

*bungei* 1827 but they appear to be slightly smaller. Although several of the chromosome types in the 16-chromosome race may be represented more than twice, only two satellited chromosomes were seen and M. Navashin, from whom the material was obtained, has not seen

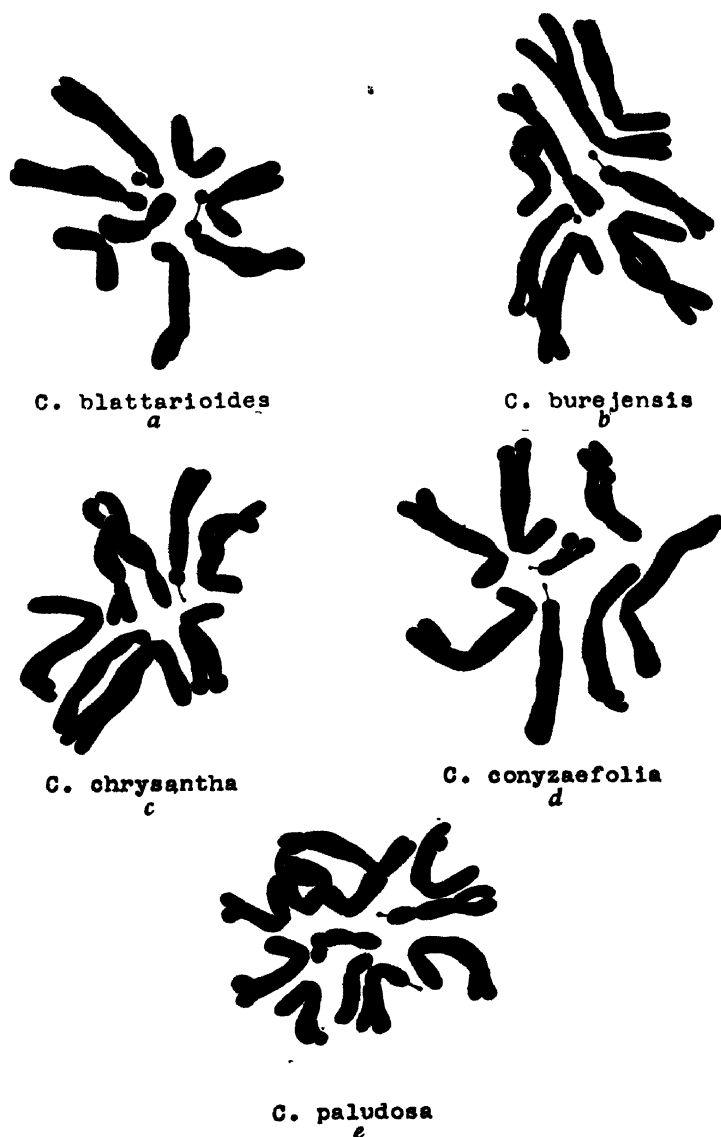


Figure 8.

more in similar material (unpublished observations). Plants of these two accessions are now in flower. Although 1827, with 8 chromosomes, is more nearly typical of the species in shape of leaves and habit of branching, yet the flower-heads and finer details of the

inflorescence are very similar in the two. Apparently 2174, with 16 chromosomes, is a form of this species resulting from some sort of chromosomal variation, the precise nature of which can be determined only by further study.

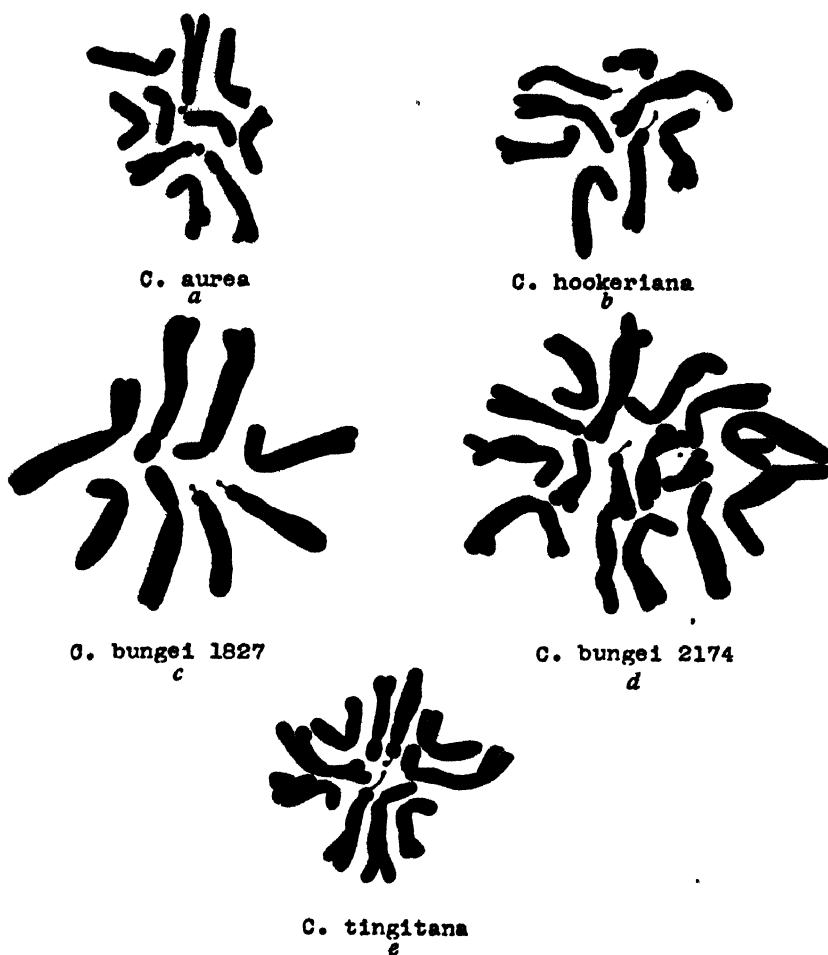


Figure 9.

*C. tingitana* ( $2n=10$ , fig. 9e) has chromosomes resembling those of *C. asturica* but differing markedly from them in one chromosome pair.

### **Eucrepis**

The chromosomes of *C. leontodontoides* ( $2n=10$ , fig. 10a) and *C. multicaulis* ( $2n=10$ , fig. 10b) are noticeably different in size, those of *C. multicaulis* approaching those of *C. asturica*, those of *C.*

*leontodontoides* being much smaller. Both complexes differ in other details from that of *C. asturica* and from each other.

*C. tectorum* ( $2n=8$ ), *C. neglecta* ( $2n=8$ ), *C. parviflora* ( $2n=8$ ), *C. capillaris* ( $2n=6$ ), and *C. nicaeensis* ( $2n=8$ ) do not particularly

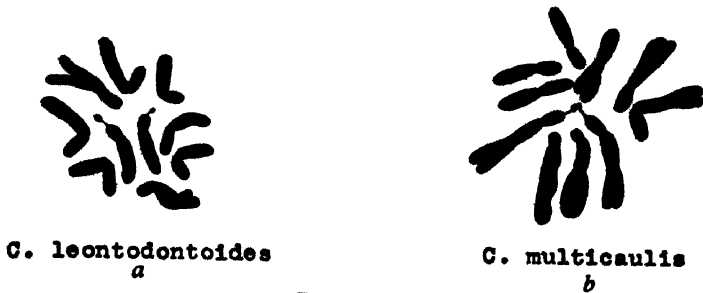


Figure 10.

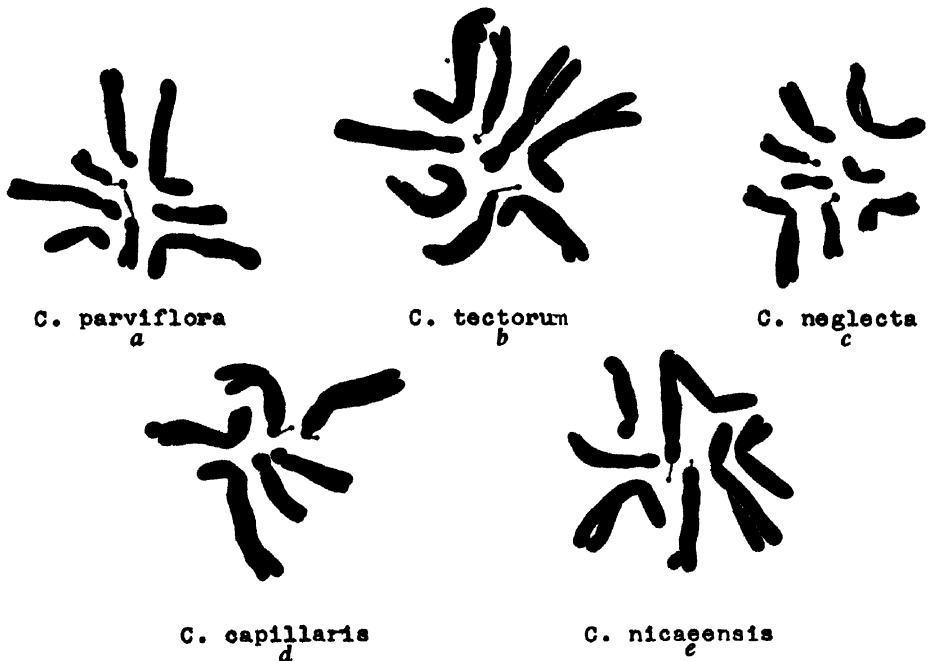


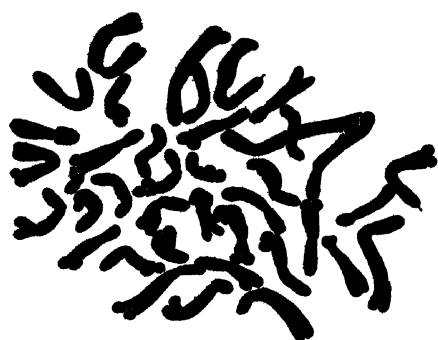
Figure 11.

resemble each other in chromosome morphology (fig. 11). Those of *neglecta* are the smallest, those of *tectorum* the largest of this series.

In *C. biennis* the chromosome number has been variously reported as  $n=16$  (Marchal, 1920),  $n=20$  (Rosenberg, 1918),  $n=21$ ,  $2n=42$  (Rosenberg, 1920), and  $2n=40$  (Mann, 1925). Investigations of several plants (table 1) have shown that there is an actual variation in number from plant to plant and this is confirmed by



unpublished observations of M. Navashin (cf. Collins, Hollingshead, and Avery, 1929). Counts of 39 and 41 have been obtained and the plant from which the plate in figure 12a was drawn had 39 chromosomes. Satellites have been seen on chromosomes of at least two different types but those on only one type were visible in this figure.



*C. biennis*  
a



*C. ciliata*  
b

Figure 12.

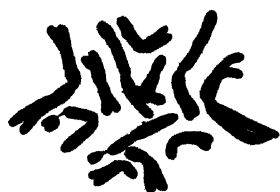
A similar situation with respect to variation in number was found in *C. ciliata* (fig. 12b) the two plants examined having 40 and 42? chromosomes respectively. The latter count is not well established but there were more than 40 chromosomes. Again satellites were seen but the large number of chromosomes precluded a



*C. mollis*  
a



*C. lyrata*  
b



*C. montana*  
c



*C. hierosolymitana*  
d

Figure 13.

decision as to their exact number. The chromosomes of *C. biennis* and *C. ciliata* are of the same size order.

The group of four species with  $2n=12$  (fig. 13), *C. mollis*, *C. lyrata*, *C. montana*, *C. hierosolymitana*, gave material on which it was somewhat difficult to make out exact details of chromosome morphology. The chromosome outlines were not quite so clear as in most species and the increased number added to the difficulty. True satellites were found in *montana* and distal constricted ends which often resembled satellites were found on V-shaped chromosomes of *lyrata*, *mollis*, and *hierosolymitana*. Whether there are other small true satellites has not been definitely decided.

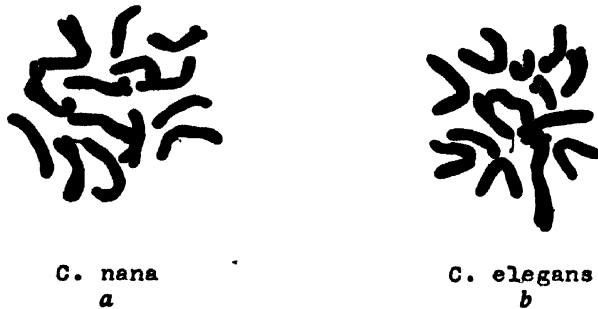


Figure 14.

The material of *C. nana* and *C. elegans* ( $2n=14$ , fig. 14a, b) was limited to a few root tips from germinated seeds and the figures available were anything but satisfactory for a study of chromosome morphology though the number was quite clear. The complexes are similar, containing long and medium V's and probably a satellited pair of chromosomes with submedian constrictions. In each of the figures reproduced only one satellite appears, but in *C. nana* other plates were seen containing two satellites.

Extensive study of material from six plants of *C. tenuifolia*, grown from seed collected from the wild, revealed always a somatic number of fifteen chromosomes. Two of these plants have flowered with the help of artificial light and one produced a number of achenes. Two plants which grew from these achenes were examined and each had 15 chromosomes. Reduction divisions of the plants which flowered were very irregular showing sometimes 15 univalent chromosomes and sometimes both bivalents and univalents. An examination of the somatic chromosomes (fig. 15a) shows no good evidence of hybrid origin since many of the chromosomes have seemingly morphological mates. In the plate drawn, the upturned chromosome lying beneath



*C. tenuifolia*  
a



*Pterotheca sancta*  
b

Figure 15.

another in the central region is probably similar to the short one in the middle lower portion of the figure. This was the only plate observed in which there appeared to be 4 satellited chromosomes although 3 were seen very commonly. Whatever the origin of this species the evidence points to some form of parthenogenesis or apogamy as the usual means of reproduction. This would reconcile the irregularity in reduction divisions and low proportion of good pollen



*C. dioscoridis*  
a



*C. pannonica*  
b



*C. lacera*  
c



*C. chondrilloides*  
d

Figure 16.

with the apparently constant odd number of chromosomes and the fairly high proportion of achenes obtained.

*Pterotheca sancta* ( $2n=10$ , fig. 15b) has chromosomes of the *C. foetida* type. We are indebted to Dr. M. Navashin for kindly giving us the opportunity of reproducing the figure from his slide. In this, the only plant examined, apparently one of the short chromosome pairs was heteromorphic, the spindle-fiber attachment of one chromosome being median, and of its mate being submedian.

*C. dioscoridis*, *C. pannonica*, *C. lacera*, and *C. chondrilloides* (fig. 16) have eight chromosomes in garnitures which resemble each other and those of *C. burejensis*, *C. chrysanth*a, and *C. conyzaefolia* of the



*C. incana*

Figure 17.

subgenus **Catonia** (above). The chromosomes of *dioscoridis* seem to be slightly smaller and those of *pannonica* slightly larger than those of the other species in this group. *C. incana* ( $2n=16$ , fig. 17) is probably a tetraploid species since it has 4 similar chromosomes in each set. The chromosomes resemble those of the group just described.

The complexes of *C. reuteriana*, *C. praemorsa*, *C. incarnata* and *C. palaestina* (fig. 18), and of *C. pulchra* and *C. gymnopus* (fig. 19), with 8 large chromosomes, are similar, those of *C. incarnata* and *C. praemorsa* being probably indistinguishable. Each of these species is characterized by the presence of one pair of long V-shaped chromosomes. Each has a pair of very small satellites. On the shortest chromosome pair of *C. pulchra* in well fixed, darkly stained material there could be found very small distal satellites. These have not been seen in other species. This pair of chromosomes differed in shape



*C. reuteriana*  
*a*



*C. praemorsa*  
*b*



*C. incarnata*  
*c*

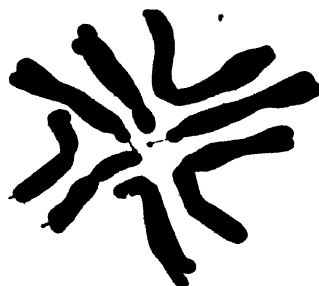


*C. palaestina*  
*d*

Figure 18.



*C. pulchra* (1)  
*a*



*C. pulchra* (2)  
*b*



*C. pulchra* (3)  
*c*



*C. gymnopus*  
*d*

Figure 19.

in the strains of *pulchra* examined. In one strain (1213, fig. 19c) a plant occurred in which the attachment constriction of one of the members of the pair was nearly median, in the other member it was distinctly nearer the proximal end. In another plant of the same strain (fig. 19b) in both members of the pair the constriction was median. In plants of two other strains (1483 and 1894) each member of the pair had the constriction nearer one end of the chromosome (fig. 19a).

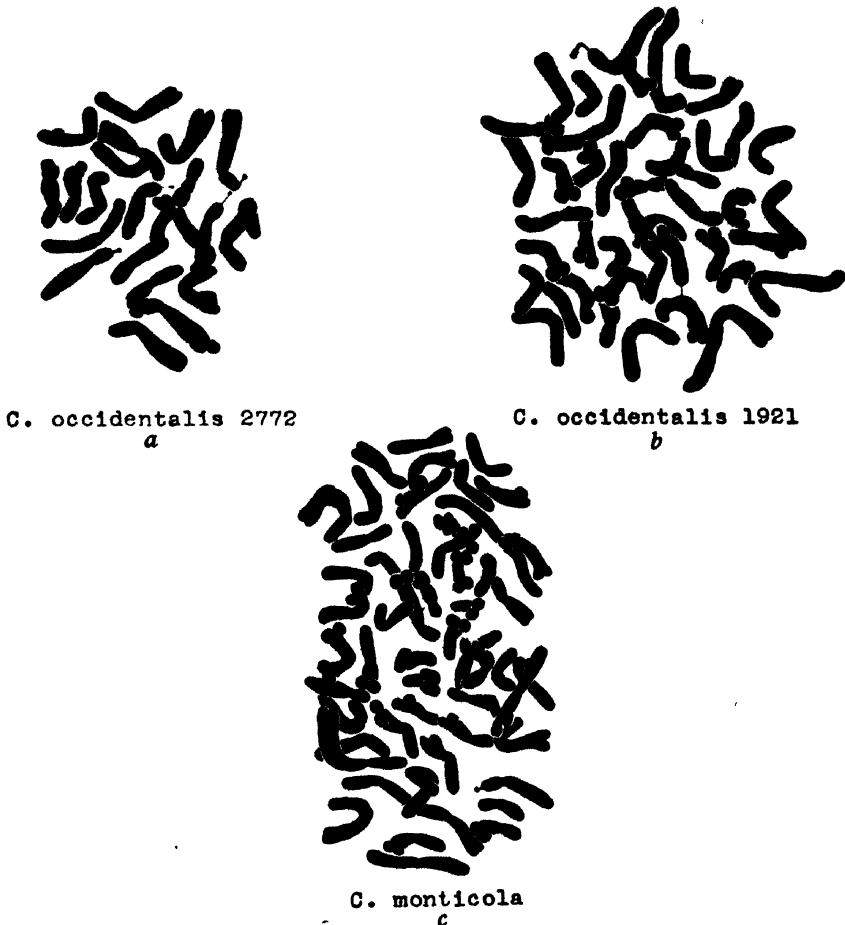


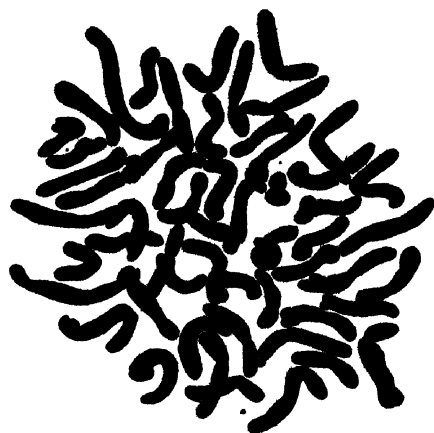
Figure 20.

The chromosomes of the American species (except *C. nana* and *C. elegans*) (figs. 20 to 22) are intermediate in size and the various species show similar types of chromosomes. Satellites have been seen in nearly every species but only in some of the 22-chromosome forms was it possible to determine their number, 4 satellited chromosomes having been established in several cases.

The *C. occidentalis*, *monticola*, *scopulorum* and the *C. gracilis*, *acuminata*, *barbigera* groups (figs. 20 and 21) show polyploid series with a base number of 11 (table 1). In some cases, indicated by (?) after the chromosome number, the large number of chromosomes or paucity of material has prevented an exact determination but the number



*C. acuminata* 1778  
a



*C. acuminata* 1830  
b



*C. gracilis* 2572  
c



*C. barbigera* 1842  
d

Figure 21.

given was the most likely one. In *C. occidentalis*, *C. gracilis*, and *C. acuminata*, variation in number between different forms has been found. The occurrence of the odd numbers 33 and 55 point to natural crossing and there is abundant morphological evidence that natural crossing, even between species, is no uncommon occurrence. The occurrence of the numbers 33 and 44 in typical *C. acuminata* suggests

the possibility that the somatic number 22 exists in this species, too, as it does in *occidentalis* and *gracilis*.

The *C. andersoni*, *glauca*, *runcinata* group (fig. 22) of which several plants representing different accessions (table 1) have been

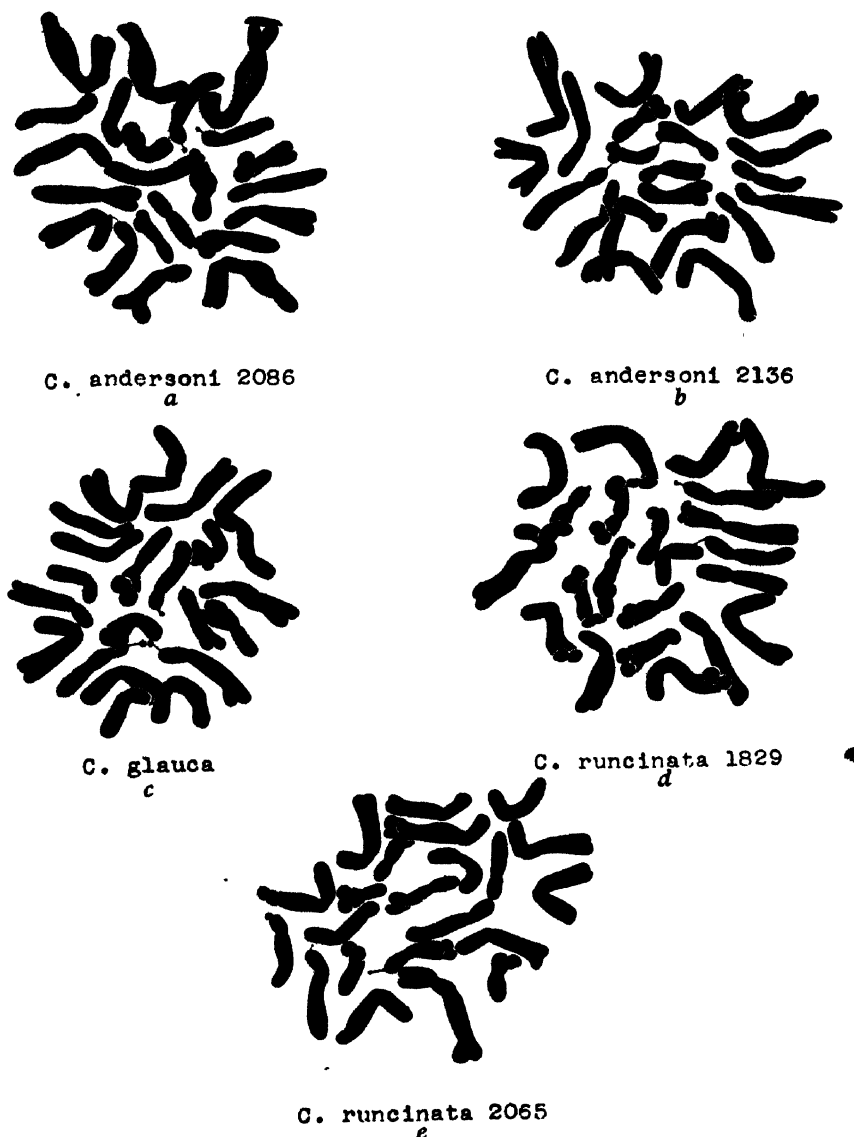


Figure 22.

examined, has given uniformly complexes of 22 chromosomes containing similar types. Whether it would be possible by a prolonged study to reveal differences in chromosome morphology between these species cannot be said.



*Ixeris graminea* ( $2n = 16$ ), *C. bulbosa* ( $2n = 18$ ), and *C. japonica* ( $2n = 16$ ), while apparently representing the three large subgenera in certain morphological features, have chromosomes whose morphology has much in common and which differ from the other species described so noticeably that they are grouped together for descriptive purposes (fig. 23). The general features in which they differ, size and sharpness of outline, have been noted earlier. In each species the shapes of most of the chromosomes suggest median or submedian fiber attachments. At least one pair of chromosomes in each bears satellites or structures resembling them.



Figure 23.

## THE PHYLOGENY OF SIXTY-SEVEN SPECIES OF CREPIS

The phylogenetic relations between the four subgenera, as at present understood, are fairly well represented by the four major divisions of the chart, figure 24, in which **Paleya** occupies the center, **Barkhausia** the upper right portion, **Catonia** the upper left, and **Eucrepis** the lower half. The relations between the species in each subgenus are indicated by the arrangement in groups and by lines leading to or toward the region of origin in the center. The chromosome number of each species is also given but in order to compare chromosome morphology it will be necessary to consult the figures presented in foregoing pages.

### **Paleya**

**Paleya** is certainly the most primitive of the four subgenera. It contains only four or five species which are all perennials of restricted distribution but which occupy very widely separated areas (south-western Europe, Abyssinia, and western Himalaya). In these species, unlike other *Crepis*, the involucre is not clearly differentiated into outer and inner series of bracts, at least as regards length, although

in one species the outer bracts differ in color from the inner ones. The leaves are large and nearly entire and the heads are few and large. The achenes are large, elongate, and in three or four of these species they are more like **Barkhausia** achenes while in the other they are more like those of **Catonia**. *C. asturica* is one of those with **Barkhausia**-like achenes and the marked similarity between its chromosomes and those of *foetida* and its close relatives has been noted. It seems reasonable to assume that **Paleya** was formerly a widespread group containing more numerous species than at present and that among these species there existed primitive forms of all three of the other subgenera. As most of these primitive forms are now extinct, evidence is lacking for direct connection between **Paleya** and some **Catonia** species and between **Paleya** and all **Eucrepis** species.

### **Barkhausia**

Of the other three subgenera, **Barkhausia** exhibits its relationship to **Paleya** most obviously both in external morphology and in chromosomes, but it must be remembered that as yet the chromosomes of only one species of **Paleya** have been seen. **Barkhausia** contains about one-fourth of the species under discussion; these occur in portions of Europe, Asia, and Africa bordering on the Mediterranean. A few of these species are of wide distribution within the area defined, but most of them are of rather restricted range. The species of **Barkhausia** are all characterized by having definitely beaked achenes in which the beak is usually equal to or longer than the body of the fruit. This specialization for seed dispersal indicates considerable advancement beyond **Catonia** and **Eucrepis**, but, as was noted above, the line of development is well advanced in most of the present species of **Paleya**. There is also present in **Barkhausia** a strong tendency to have the marginal achenes different from the inner ones either in shape or in color and texture of the pericarp or in both respects, and in some species, like *alpina*, *aspera*, and *aculeata*, these differences in outer and inner achenes are very striking. Although a few species of **Eucrepis** have strikingly modified marginal achenes and it is not uncommon in both **Eucrepis** and **Catonia** to find the marginal achenes slightly different in shape from the inner ones, yet in neither of these subgenera is found as great specialization of the marginal achenes as occurs in **Barkhausia**. Nearly all species of **Barkhausia** are annuals which may also be taken as evidence of more recent development than in **Catonia** and most **Eucrepis**. There is much variation among these

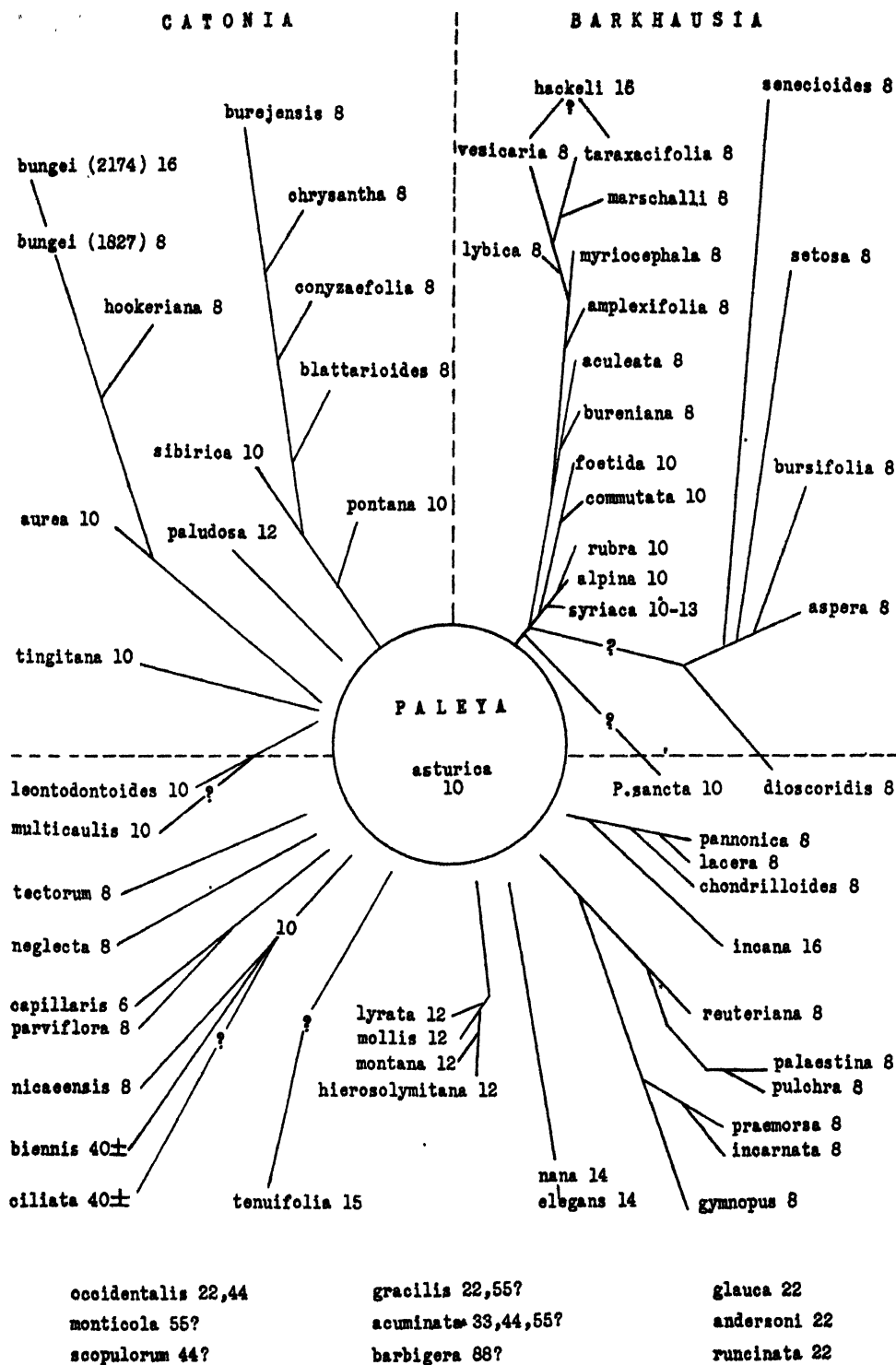


Figure 24.

EUCREPIS

species and within some of them, in leaf characters, number and size of heads, and size of achenes.

*Anisoderis*, a subgroup of **Barkhausia**, contains the species most resembling **Paleya**. This subgroup originally contained *C. alpina*, *rubra*, and *foetida*, and to these may be added two species now known as *C. alpina* var. *syriaca* and *Rodigia commutata*. The close resemblance between the chromosomes of these species and those of *C. asturica* has been noted. All the other species of **Barkhausia** have 8 (or 16) chromosomes. Their arrangement in the chart, figure 24, is based primarily on external morphology, particularly on achene characters, and the achenes of some of them have been illustrated in an earlier paper (Babcock and Lesley, 1926). Although *aspera* and *aculeata* are rather closely related, their very different achenes show their relationship to different species. Thus *aspera* most nearly resembles *dioscoridis* (of **Eucrepis**) in its marginal achenes, while its inner ones are more like those of *bursifolia*, *setosa*, and *senecioides*, but these five species are all widely separated from one another in other characters. It may be that the *aspera* line and the *aculeata* line arose from a common stock of distinct origin from the *alpina* line, the supposed progenitor being some (extinct?) species of **Paleya** having 8 chromosomes. For present purposes, however, it is more convenient to indicate these two lines as arising from the *alpina* line.

The four species, *bureniana*, *aculeata*, *amplexifolia*, and *myriocephala* differ from the remaining five species, grouped near the top, in having the marginal achenes distinctly different from the inner ones. *C. bureniana* most nearly resembles *foetida* in external morphology, while the divergence is progressive until in *myriocephala* one finds a highly specialized species with very numerous, small heads and many small fruits. The other eight chromosome species of this line are all closely related to *myriocephala* but are not so highly specialized through reduction in size of heads, flowers, and fruits. *C. lybica*, indeed, has the largest heads and fruits of the six species, although its chromosomes are the most like those of *myriocephala*. This may be explained, however, as the result of genic mutations unaccompanied by marked changes in chromosome morphology. In *C. hackeli* the evidence from external morphology is in fair agreement with the cytological evidence that this is a tetraploid or amphidiploid species.

Fig. 24. Phylogenetic chart of sixty-seven species of *Crepis*. The subgenus **Paleya**, center, is the most primitive. In the other three subgenera, **Barkhausia**, upper right; **Catonia**, upper left; and **Eucrepis**, lower half, the degree of relationship is roughly indicated by connections or absence of connections with **Paleya**.

*Ixeris graminea* probably should not be included in *Crepis*. Although its achenes are more like those of **Barkhausia** than they are like those of the typical *Ixeris* of Cassini, yet in other details of external morphology it is certainly closer to *Ixeris* and *Lactuca* than to *Crepis*. The evidence regarding the chromosomes provides assistance at this point. Although the number happens to be 16, the chromosomes are very much smaller than in any species of **Barkhausia** and in size and appearance they closely resemble those of *C. bulbosa* and *C. japonica* which are mentioned under **Catonia** and **Eucrepis** respectively.

### **Catonia**

**Catonia** has less obvious connection with **Paleya** than has **Barkhausia**, yet the evidence from external morphology as well as from chromosomes strongly indicates such an origin. The existence of one species of **Paleya** (*C. oligocephala* Sch. Bip.) with **Catonia**-like characters is especially significant. Unfortunately the chromosomes of this species have not been seen. **Catonia** includes slightly less than one-fourth of the species thus far investigated cytologically and these are all perennials of the Old World. They are distributed in restricted areas scattered throughout Europe, Asia, and northern Africa; the subgenus is of much wider distribution than **Barkhausia**. All of these species have achenes resembling those of *C. oligocephala* more or less, but some of them are sufficiently different in their achenes and in other characters to necessitate the assumption of more than one prototype. Two principal lines are indicated in the chart, therefore, also two minor groups in each of which only one species has been examined cytologically.

*Crepis sibirica* exhibits closest resemblance to *C. oligocephala* in external morphology and the other species of this line are progressively distinct from the supposed prototype. The second group, *aurea*, *hookeriana*, and *bungei*, are so distinct from *C. oligocephala* as to necessitate the assumption that they arose from some other species. *C. tingitana*, on the other hand, resembles *aurea* in certain respects although very distinct in others; it does not seem improbable that the two arose from a common prototype. *C. paludosa* resembles *sibirica* and its allies in habit and leaves but the much smaller heads, and especially the very distinct achenes, make it necessary to assume a separate origin for *paludosa*.

*Crepis bulbosa* has long been recognized as one of the most outstanding species of the genus. In fact it has been referred to five

other genera, including *Aetheorhiza* of Cassini, who treated it as a monotypic genus. It is the only species of *Crepis* thus far examined having 18 chromosomes, and, as in *Ixeris graminea*, the chromosomes are very small. These facts would appear to warrant the reinstatement of Cassini's genus.

### **Eucrepis**

These species not only show the least morphological resemblance to those of **Paleya** but they also comprise the most heterogeneous of the four subgenera. There is general resemblance within the subgenus, however, in the more numerous and smaller heads, the marked reduction of the outer involucre bracts, and the mostly smaller, beakless achenes. Of the thirty-eight species thus far studied cytologically, about one-third are annuals or biennials and two-thirds, perennials. This subgenus is the most widespread in geographic distribution, being represented in North America as well as throughout the Old World north of the equator. As is indicated in the chart, there are eight groups of obviously related species and some half-dozen species which are distinct from each other and from the several groups.

*Crepis leontodontoides* and *C. multicaulis* are sufficiently similar to the **Catonia** species, *tingitana* and *aurea*, to suggest a common origin. The geographic distribution of three of these species is not inconsistent with such a view. *C. multicaulis*, however, is widely separated from the other three geographically. At the same time it is among the most outstanding species in the genus because of extreme reduction in size of inflorescence although it is perennial like the other three. It seems probable, therefore, that *multicaulis* sprang from the same ancestral stock as the other three but is a more recent species and that there were several connecting forms which have disappeared.

The two annuals, *parviflora* and *capillaris*, are obviously close in external morphology and occupy different adjacent geographic areas. On the basis of specialization through reduction in size of flowers and fruits *parviflora* seems to be more recent and this view would be consistent with the chromosomal evidence if fragmentation of one of the *capillaris* chromosomes and marked changes in the others could be assumed. Fairly close to these are two other annuals, *neglecta* and *tectorum*, yet both are so different in their chromosomes as to require the hypothesis of a separate origin from each other and from the *capillaris* group. Both *tectorum* and *neglecta* exhibit a marked tendency to the production of a beak on the achene, but this is true also in certain forms of *leontodontoides*. It would appear that these

are really intermediate between **Eucrepis** and **Barkhausia** and that in course of time they may develop definitely beaked achenes.

Another group includes *nicaeensis*, which is found to be either annual or biennial, and *biennis*, which is usually biennial but sometimes blooms the first year. The close resemblance of the two species has been emphasized by Bischoff (1851), yet one has 8 chromosomes of medium size and the other about 40 rather smaller ones. It was deemed necessary to assume a common ancestral form with 10 chromosomes because of the evidence from interspecific hybrids (Collins and Mann, 1923; Collins, Hollingshead, and Avery, 1929) that *C. biennis* is an octoploid species. *Crepis ciliata* may also have derived its large number of chromosomes through polyploidy, but morphologically it is too distinct from *biennis* and *nicaeensis* to warrant the assumption of a very recent connection.

*Crepis tenuifolia* is a very outstanding species of **Eucrepis** not only because of its peculiarities of external morphology but also on account of its odd number of chromosomes discussed above. The fact that it appears to produce seed apomictically suggests the possibility that it originated as a hybrid between two species with 14 and 16 chromosomes respectively, although, as has been pointed out, the shapes of the various chromosomes offer no good evidence for this method of origin.

The four species with 12 chromosomes are very distinct from one another, but have quite similar heads, flowers, and fruits, and they are all perennials. *C. mollis*, with its nearly entire leaves and few, rather large heads, appears to be the least specialized; the other three are about equally advanced and are correspondingly less widely distributed.

*C. nana* and *elegans* form a unique group in external morphology, in geographic distribution, and in the number of their chromosomes. *C. nana* is the most widely distributed species in the genus. It occurs sporadically from Turkestan to northeastern Siberia and in North America from western Alaska to northwestern Canada, in Labrador, the Rocky Mountains, and the Sierra Nevada. *C. elegans* occurs in Alaska and the Rocky Mountains. There is good morphological evidence that *elegans* has been derived from *nana* and the extensive boreal and alpine distribution of *nana* indicates that it is either a much older species or a more successful species, perhaps both. There are no close relatives among the other species which have been examined cytologically.

Of the six species, *reuteriana*, *praemorsa*, *incarnata*, *gymnopus*, *pulchra*, and *palaestina*, the first four are perennials and the other two annuals. *C. praemorsa* and *C. incarnata* are very closely related although fairly distinct from each other morphologically, and still more distinct from their near relative, *gymnopus*. Similarly *pulchra* and *palaestina* are obviously related although still more distinct from each other. *C. praemorsa*, *incarnata*, and *gymnopus* bear less resemblance to *reuteriana*, however, than do *palaestina* and *pulchra*; they probably diverged from the *reuteriana* line considerably earlier than did *palaestina* and *pulchra*. Of the last two, *pulchra* is more highly specialized through reduction in size of flowers and its high self-fertility.

*Crepis pannonica*, *lacera*, and *chondrilloides* are obviously closely related perennial species and they probably stand in the above order in their phylogenetic relations to one another. This is indicated by differences in degree of dissection of the leaves. In *pannonica* the leaves are nearly entire; in *lacera* they are deeply dissected; while in *chondrilloides* they are extremely finely dissected. The geographic distribution is in agreement with this relation, since *pannonica* occurs from Hungary to Turkestan, *lacera* occurs only in central and southern Italy, and *chondrilloides* is restricted to a small region in Istria.

*Crepis incana* represents a group of perennial species which is closely related to the foregoing although very distinct. It was probably derived from the same ancestral stock.

*Crepis dioscoridis* bears the evidence of its relationship to *C. aspera* (of **Barkhausia**) in its peculiar marginal achenes and annual habit. If it arose from the same stock as *aspera* it must have diverged at a rather remote time. It is possible, of course, that this similarity in the marginal achenes is merely a case of parallel variation but if such is the case the ancestry of *dioscoridis* is still more obscure. Although it bears some resemblance to the *pannonica* group, especially in appearance of the involucre and in the morphology of its chromosomes yet in most respects it is very distinct from those species.

*Pterotheca sancta* seems to belong in *Crepis*, not only on the basis of its somatic chromosomes, but also on morphological grounds, the details of which cannot be discussed here. The origin of this species, however, is quite as uncertain as that of *dioscoridis* or *tenuifolia*. Its possible derivation from the **Barkhausia** stock is suggested by the resemblance of its chromosomes, but morphologically it seems quite as probable that it had a common progenitor with the *reuteriana* group.



The American species which have been examined cytologically, excepting *elegans* and *nana*, comprise a unique group with a different basic chromosome number ( $n=11$ ) from any other *Crepis*. None of them occurs east of the Mississippi River and the center of distribution is in southwestern Canada and northwestern United States. This suggests the probability of an Asiatic origin, but thus far no oriental species has been found with  $n=11$  chromosomes. Neither have any oriental species been found which closely resemble any of these American species. A few Asiatic species are known, however, which resemble certain American species in some ways and it may be shown eventually that they arose from a common prototype.

Although the problem of the phylogenetic relation of these American species to other *Eucrepis* is unsolved, certain assumptions may be warranted on the basis of chromosome numbers, external morphology, and ecology. All nine species are perennials. Three of them, *runcinata*, *glauca*, and *andersoni*, occur in moist meadows and alkali bogs and have fleshy rootstocks. The other six occur mostly in dry, rocky or gravelly places and have very long, slender, woody rootstocks. The first three are equally diverse from the other six in leaves, heads, and achenes. Thus there are two very different subgroups of American species and the situations with regard to chromosome numbers are equally diverse. Although fourteen different forms of *runcinata*, *glauca*, and *andersoni* have been examined, they all have  $2n=22$  chromosomes. But among the other six species, both tetraploidy and octoploidy occur as well as unbalanced polyploidy (33 and 55) accompanied by seed-bearing, which suggests reproduction by some form of apomixis. The existence of two such diverse groups in the same general region and with a common basic chromosome number suggests a common origin but divergence must have occurred at a comparatively remote period. Yet as compared with the Old World species of *Eucrepis*, these nine are probably of relatively recent origin. This would necessarily be the case if they were derived from Asiatic ancestors and it is strongly indicated by the higher chromosome numbers of this group. The probable method of origin of these remarkable groups of species is that first suggested by Winge (1917) and later confirmed experimentally by Clausen and Goodspeed (1925). This method consists of interspecific hybridization followed by delayed mitosis in the fertilized egg cell or in some initial cell of a shoot, during which the chromosomes all divide without passing to the poles. Thus a complete diploid set of chromosomes from

both parents is provided after which mitosis proceeds regularly. Some such amphidiploid hybrids are known to be vigorous and fully fertile. It is only necessary to assume natural crossing between two species with  $n=7$  and  $n=4$  chromosomes, followed by amphidiploidy, to explain the origin of species with 22 chromosomes. Because of the diversity between the two groups of American species, however, it seems necessary to assume that at least three different species were involved in the original crosses. Some of the other numbers which occur in the **Eucrepis** series may also have originated through amphidiploidy.

*Crepis japonica* (*Youngia lyrata* Cass.) is the type species of Cassini's genus. As in the case of *Ixeris graminea* and *Aetheorhiza bulbosa*, the chromosomes are much smaller than *Crepis* chromosomes. This evidence, together with that from external morphology and geographic distribution, will probably be considered sufficient to warrant the reinstatement of Cassini's *Youngia* as that genus was originally defined.

Summarizing briefly with respect to subgeneric relationship, **Paleya** is a small group of species which appear to be more primitive than all other *Crepis* at least in certain characters. There are two distinct subgroups of **Paleya**, viz., species with **Barkhausia**-like achenes and one species with **Catonia**-like achenes. **Barkhausia** contains species which show strong resemblance to certain **Paleya** species, although certainly more highly specialized. At the same time, within **Barkhausia** are found the highest degree of specialized structure in the beaked achenes and the largest amount of adaptation through development of the annual habit. The combination of these two lines of specialization, however, while advantageous in particular environments, has not resulted in wide distribution of the subgenus as a whole. This restriction of adaptation accompanying marked specialization must be considered along with the evidence that many species of **Barkhausia** appear to be of recent development. In **Catonia** is found a group of perennial species some of which exhibit evidence of relationship with an existing species of **Paleya** while for others it is necessary to assume other prototypes. The species of **Catonia** are less highly specialized than those of **Barkhausia** and **Eucrepis**, and most of the species are of comparatively restricted distribution. **Eucrepis**, the largest, most widely distributed, and most heterogeneous division, exhibits connections with both **Barkhausia** and **Catonia** in certain of its species but there is no evidence, among the species dis-

cussed in this paper, of close connection with *Paleya*. It seems most probable that the prototypes of *Eucrepis* and the present or earlier species of *Paleya* arose from a common stock subsequent to its differentiation from closely related genera such as *Hieracium* and *Lactuca*.

## CHROMOSOMES AND PHYLOGENY

The cytologist or geneticist cannot fail to have noticed in the literature of recent years an increasingly close relationship between the study of the chromosomes and of external morphology with a view to classification. The impression is rather strong that in most cases it is the cytologist or the geneticist who has realized how useful or in some cases how fundamental his findings are to the elucidation of phylogenetic problems, and so has been led by a combined study of chromosomes and external morphology to formulate classification systems and phylogenetic hypotheses or to criticize those based on external morphology alone. It is to be hoped that taxonomists will come to the same realization and that in the future it will be considered as desirable to know the nature of the chromosomes of any plant as it is to be familiar with the details of its external morphology, particularly in cases where critical decisions are necessary.

Among the most outstanding cases where a knowledge of chromosome numbers has been useful in classification is that of the genus *Rosa*. Hurst (1925) utilized the cytological findings of Täckholm (1920, 1922) and Blackburn and Harrison (1921) in the classification of this genus, which in spite of the life-long labors of several botanists was still in an admittedly unsatisfactory state. These investigations had shown that there was in the genus a multiple series of chromosome numbers with a base number of seven. With this in mind a workable system was formulated, the most outstanding feature of which was that the whole *Caninae* section was characterized at reduction division by a condition often seen in hybrids, viz., the presence of univalent as well as bivalent chromosomes.

Quite different but equally interesting results were obtained by Heilborn (1924) in *Carex*. When the chromosome numbers were arranged according to the taxonomic relationship within the genus groups of adjacent numbers were found, nearly related species having numbers of about the same magnitude. In the genus *Triticum* a remarkable agreement in classifications based on morphology, resistance to disease, serological reactions, and chromosome numbers is

evident (Sax, 1921, 1923). Clausen (1927) found that *Viola* species of the same systematic subgroup belonged as a rule to the same series of chromosome numbers and in the *Melanium* section of the genus he made a new subdivision on the basis of chromosome numbers. He believes his investigations afford further proof of the great importance of cytology as an aid to taxonomic research.

It is the intention of the writers to do no more than point out a few of the many instances in which a knowledge of chromosome numbers has either aided in the making of a classification system or has added weight to one already made. It is true that in some instances a study of chromosome numbers has not helped greatly in classification, for species widely different morphologically may have the same chromosome number, as in *Crepis*. When such is the case this study has shown that a knowledge of the morphology of the chromosomes may be very illuminating. The chromosome number 8 occurs in thirty-two out of the seventy species discussed and in three of the four subgenera. The possibility is good that it occurs also in **Paleya**. But a comparative study of chromosome morphology has shown that these species with the same chromosome number are usually characterized by very different chromosomes.

A study of chromosome number and morphology in relation to classification was carried out by Sveshnikova (1927) in thirty species of *Vicia*. Three main groups—**Ervum** with 14 chromosomes in all species, **Cracca** with 12, 14, 24, and 28, and **Euvicia** with 12 and 14—were represented in the study. The chromosomes were divided into four groups according to the relative lengths of the arms and the presence of satellites and each species was described on this basis. When the species were arranged in groups according to the numbers of chromosomes of different classes, the classification was in general agreement with that usually applied by systematists. Indeed this investigator was able to make a key based on chromosome number and morphology which corresponded very nearly with one worked out by Ascherson on external morphology.

Wexelsen (1928), however, found no such parallelism in the differentiation of chromosome complexes and external morphology in his studies on chromosome numbers and morphology in eighteen species of *Trifolium*. Species which were far removed taxonomically and very different in morphology had very similar chromosome complexes, and very nearly related species had very different complexes. This he believes presents a very clear demonstration of parallel vari-

ation, for example the presence of one pair of satellited chromosomes in many species would be due to independent parallel mutations and not to the fact that they have been derived from a common source.

*In most instances in Crepis similarity of chromosomes is most certainly associated with a common phylogenetic origin*, although it may be necessary in one outstanding case at least to assume parallel variation to account for the similarity of the chromosomes of species far removed phylogenetically. We refer to the resemblance of the chromosomes of the *conyzaefolia*, *chrysantha*, *burejensis* group of **Catonia** to those of the *pannonica*, *lacera*, *chondrilloides* group of **Eucrepis**.

It is interesting to note what kinds of changes with respect to chromosome number and morphology the phylogenetic chart in figure 24 involves. The first difficulty we are faced with is the fact that only one species of the subgenus **Paleya**, which is believed to contain or to have contained the progenitors of the species of the other subgenera, has been examined cytologically. It seems probable that, since each of the other subgenera shows variation between species in the number of chromosomes, the same would be found to occur here. For the two lines (one in **Barkhausia**, one in **Catonia**), however, where the connection with **Paleya** is clearest ten is apparently the most primitive number. In each line one or more reductions to eight have occurred. This is true also of the second main line of **Catonia**.

The 8-chromosome species of **Eucrepis** could be assumed to have arisen from 8-chromosome **Paleya** species not examined cytologically or now extinct. If so it is necessary to assume that within **Paleya** a reduction from 10 to 8 or an increase from 8 to 10 chromosomes has occurred during the evolution of the species of this subgenus. Within **Eucrepis** the relations depicted in the chart (fig. 24) involve a reduction to 8 from the supposed 10-chromosome progenitor of *nicaeensis* and possibly a reduction to 6 from an 8-chromosome progenitor of *capillaris*.

The two known methods by which a reduction in number of chromosomes may take place are (1) the elimination of a pair of chromosomes following irregularities in meiosis and (2) the fusion of non-homologous chromosomes. Examples are known where plants possess one pair of chromosomes less than the normal diploid set as in *Triticum* (Kihara, 1924, 1925), *Avena* (Huskins, 1927), and *Primula kewensis* (Newton and Pellew, 1929), but the species in question are admittedly of polyploid origin and the plants are partly or wholly

sterile. In other genera where monosomic plants are known (for example, in *Datura* and *Nicotiana*), which would be expected to give progeny lacking one chromosome pair, such progeny have not been reported. Moreover, M. Navashin's extensive study (1926, 1929) which included several thousand plants of three species of *Crepis*, failed to find a plant with even one chromosome missing. In view of these facts the explanation of reduction in chromosome number by elimination of a pair is, to say the least, questionable.

Similarly, evidence is lacking in this genus that a decrease in number could have resulted from end to end fusion of non-homologous chromosomes. M. Navashin found no instance of it in his investigations and neither does a study of the chromosomes of the various species in which a reduction is supposed to have occurred show any evidence of such a process. To take an extreme case, the 8 chromosomes of *senecioides*, presumably derived from a 10-chromosome stock, are on the whole smaller than those of the supposed stock from which they have sprung and certainly no one of them is long enough to represent simply an end to end fusion of two chromosomes of the parental stock.

It has been suggested in the animal kingdom by Wilson (1925) and Painter (1925) and in the plant kingdom by Delaunay (1926) and favorably taken up by Jaretsky (1928) that the occurrence of small chromosomes in a complex may represent an intermediate step in the process of gradual diminution in the size of a chromosome which terminates with its disappearance. Rather small chromosomes are not rare in *Crepis* although none have been found as small as those which first prompted this hypothesis.

It is also theoretically possible, although not very probable in species having such low chromosome numbers, that such a reduction in number might result from hybridization between two 10-chromosome species. This necessitates the assumption that such hybrids occasionally produce functional gametes having only 4 instead of the normal 5 chromosomes. Self-fertilization in such a hybrid might rarely produce new stable forms having 8 chromosomes. Thus far the experimental evidence from interspecific hybrids is against such a hypothesis (Navashin, 1927; Hollingshead, 1930).

The phylogenetic chart involves as well the assumption of numerous instances of increase in chromosome number. In some of these cases, such as *hackelei*, *bungei* 2174, and *incana*, all with 16 chromosomes, each species is morphologically closely related to certain 8-

chromosome species. Such cases are readily explained by chromosome doubling. Perhaps in the case of *hackeli* (from external morphological evidence), and of *bungei* 2174 (from the fact that no more than two satellited chromosomes were seen) the doubling followed hybridization between two 8-chromosome species. It has been pointed out earlier that a similar explanation may account for the chromosome numbers of *biennis*, *ciliata*, and the American species with a base number of 11, and the same explanation might conceivably be applied to the origin of the species with 12 and 14 chromosomes. Such an explanation would involve the assumption that species with 6 chromosomes featured in the ancestry of each of the last two groups. This seems rather unlikely since the one species known which has 6 chromosomes is, judging by its external morphology, of comparatively recent origin. It seems more probable that they have arisen from species with lower chromosome numbers by some method other than one involving a doubling of all the chromosomes.

How to account for an increase in number of one or more pairs of chromosomes is another problem. In many instances the evidence from chromosome morphology, as pointed out by Navashin (1925), is not in accord with an explanation which supposes the simple duplication of a pair of chromosomes already present as a result of irregular meiotic behavior, as suggested by Rosenberg (1918, 1920), for many species in which an increase is supposed to have occurred show no two pairs morphologically alike. Moreover, although M. Navashin (1926) found trisomies rather frequently in two *Crepis* species he has never found tetrasomies in their progeny (unpublished data). It must be borne in mind, too, that experimental evidence has shown that the tetrasomies investigated up to the present, as in *Datura* (Blakeslee and Belling, 1924), *Avena* (Huskins, 1927), and *Triticum* (Huskins, 1928) are usually much less viable than normal diploid plants and even when equally viable as in *Nicotiana* (Clausen and Goodspeed, 1924) they do not breed true. In this connection, however, the occurrence of the numbers 10, 11, 12, and 13, with the variation probably involving only a single chromosome type, in apparently typical and quite viable plants of *C. alpina* var. *syriaca* is of great interest. One may assume that it will be possible to isolate constant 10-, 12-, and possibly even 14-chromosome races from this species. Whether the supernumerary chromosome is a relic of some hybrid ancestry, or whether it represents the phenomenon designated by M. Navashin as "novation," or whether it represents part of a normally 12-chromosome

complex are as yet matters of speculation only. Instances are known in the genus where a chromosome from one species has been added to the normal complex of another as a result of hybridization (Hollingshead, 1930) but the plants are weak and sterile. Similarly M. Navashin's plants which contained apparently new chromosomes of unknown origin were inviable and it has been pointed out above that plants lacking one pair of chromosomes (except in polyploid species) have not been found. Of the three suggested hypotheses to account for this situation in *syriaca*, the first (hybrid origin) appears to be the least improbable.

In some few instances the hypothesis of transverse segmentation can be advanced to account for increased chromosome number in this genus, as suggested by Mann (1925). This hypothesis is believed to account for an increased number of chromosomes in certain other genera, for example in *Secale* (Gotoh, 1924, and Belling, 1925) and in other cases (cf. Kuhn, 1928). M. Navashin (1926) found a *C. tectorum* plant in which one chromosome had fragmented and each part behaved as a new chromosome in mitosis. This process has been suggested (above) as having played a part in the evolution of *C. parviflora* from *capillaris*. Such a hypothesis, however, while explaining adequately chromosome lengths, fails to take into account differences in the shapes of the various chromosomes and must even in this instance have been accompanied or followed by changes in position of spindle-fiber attachment constrictions, which will be discussed later.

In addition to changes involving increase and decrease in number of chromosomes, the chart necessitates the assumption that changes in chromosome size have occurred during the evolution of the various species. In *Muscari*, Delaunay (1926) came to the conclusion that diminution in chromosome length by a slow process called by him "historiation" had occurred in the evolution of the chromosomes of the species he studied. Jaretsky (1928) favors the same process as a mode of chromosome evolution from his studies on POLYGONACEAE. Heitz (1928) recognizes changes in length of chromosomes as of fundamental importance in the evolution of chromosomes. Sveshnikova (1927) concluded that in *Vicia*, among the processes which had occurred during the evolution of the species she studied, there had been a reduction of chromatin connected with variation in external morphological features.



This study would seem to show that both increase and decrease in chromosome size have been relatively frequent occurrences in the evolution of the various species of the genus *Crepis*. Morphologically similar species usually have similar chromosomes and it is only within such closely related groups that it is safe to assume which chromosomes are descended from a common ancestor. In the *vesicaria*, *lybica*, etc., group, which have very similar chromosome complexes, it is possible to pick out *lybica* by the uniformly slightly larger size of its chromosomes. In this connection it is worth noting that Wexelsen found two varieties of *Trifolium repens* to have chromosomes of different size, one having uniformly larger chromosomes than the other. Though it is true that, generally speaking, the various *Crepis* species have "large" or "small" chromosomes, it does not follow that increase or decrease in length has similarly affected all chromosomes in a complex. This is obvious from a glance at such a complex as that of *setosa* which contains a very short pair along with comparatively long pairs of chromosomes. The evidence from *bursifolia-aspera* hybrids (Babcock and Clausen, 1929) indicates that the satellited chromosomes of these species are homologous. Most of the chromosomes in the two species are of the same size order but in this particular chromosome pair the species differ very markedly, *aspera* having long, and *bursifolia* unusually short, satellited chromosomes.

As has been noted earlier, cross-division of chromosomes is not unknown in the genus and as M. Navashin (1926) has pointed out, such a process might be followed by elimination of part of the fragmented chromosome. Such an occurrence could be supposed to account for the difference in size between apparently homologous chromosomes of different species were it not that no evidence is available to show that such a loss can take place and the individual still survive. Further, it has been shown that several authors favor a gradual change in chromosome size as the way in which chromosomes in related species come to differ from each other. Navashin (1926) pointed out that differences in satellite size between races of a single species may illustrate this kind of change and stated that the origin of new distinct species could probably be explained by the continual addition of such small changes. The fact that chromosomes of all sizes intermediate between largest and smallest are to be found in this genus would seem to offer good evidence for this theory.

Lastly, any evolutionary hypothesis must take into account changes in chromosome shape which are largely determined by the

presence or absence of satellites and the position of the spindle-fiber attachment. In this connection cases in which homologous chromosomes are different (heteromorphic pairs) are instructive. They would seem to offer evidence that rare changes in chromosome shape may occur without markedly affecting the external morphology.

Size differences in satellites on homologous chromosomes have been reported by a number of investigators (cf. Kuhn, 1928). In *Crepis*, M. Navashin found races of *C. dioscoridis* with two large satellites, with two small satellites, and with one large and one small satellite. In *Rumex scutatus*, Jaretsky found some plants in which one of the chromosomes of a pair have satellites and others in which there were no satellites at all. Such a condition could be expected to lead to a race with both members of the pair bearing satellites. The significance of such findings for evolution of chromosomes of a different type is obvious.

Heteromorphism, which involves a difference in position of spindle-fiber attachment, is well known in the animal kingdom. In plants, in addition to the instances of sex chromosomes which may differ both in size and shape, a few cases of heteromorphic autosomes have been found. In different samples of both *Vicia angustifolia* and *V. sativa*, Sveshnikova (1927) found the same chromosomes showing slightly different ratios in arm length. Two cases of heteromorphic pairs are reported in this paper, viz., *Pterotheca sancta* and *Crepis pulchra*. The case of *C. pulchra* is particularly interesting for here plants with two chromosomes of each kind and one with one of each were found. The first two of these races could, conceivably, if isolated, develop in the course of time into different species each characterized by its particular chromosomes.

Heitz (1928) holds that the primitive chromosome form is the equi-armed one and that asymmetrical shortening (or lengthening) gives rise to chromosomes with unequal arms. Equi-armed chromosomes occur in each subgenus and in many species of *Crepis*. Usually they are the smaller chromosomes of the complex, although they may be the largest as in the *pulchra*, *palaestina*, etc., group. There is no consistent evidence in *Crepis* to support Heitz's theory that such chromosomes are more primitive, for although they do occur perhaps more frequently in some of the supposedly older species (*C. asturica*, *C. foetida*) with low numbers, yet they also occur in the highly specialized *C. senecioides*.

Heitz's theory would also imply that a change in the form of a chromosome results only from a change in length of one of the arms. Sveshnikova does not state whether the chromosomes which showed different ratios of arm length in her material were the same length or not. In the cases observed by the writers, however, the heteromorphic pairs were nearly or quite the same length and it seems more probable that the change in form has resulted from a shifting of the attachment constriction rather than from a lengthening of one arm and a shortening of the other.

Summarizing, the following changes in chromosome numbers must be assumed to have occurred during the evolution of these sixty-seven species of *Crepis*, if the phylogenetic grouping proposed here be accepted. (1) Reduction in number from 10 to 8 and from 8 to 6, changes which it is difficult to explain in the light of our present knowledge. It is possible, however, that such changes might have come about through a process of gradual diminution of a particular pair of chromosomes or that interspecific hybridization or fusion of non-homologous chromosomes might have given rise to a new species with the reduced number, although there is no experimental evidence for such hypotheses. (2) Increase in number from 8 to 16, a change which may have occurred in either of two ways, viz., doubling of the  $2n$  group resulting in true tetraploidy; and doubling in a hybrid between two 8-chromosome species, resulting in amphidiploidy. (3) Increase from 10 to 40 or thereabouts (*biennis*, *ciliata*?) which according to experimental evidence must have resulted from once repeated doubling of the  $2n$  group producing octoploidy. (4) Increase from 22 to 33, 44, 55, and 88. The even numbers probably arose through chromosome doubling as described above and the odd ones may have arisen through hybridization between even numbered forms or by the fusion of somatic gametes with normal ones. (5) Increase from lower numbers to 12, 14, 15, and 22 chromosomes, which in some cases may have resulted from transverse segmentation. The higher even numbers could be explained by the assumption of amphidiploidy and the odd one could conceivably have arisen as a result of hybridization, though again experimental evidence for such an origin is lacking. In addition to changes in number many changes in chromosome size apparently have occurred and to the present writers it seems probable that most of these changes have taken place gradually during the phylogenetic differentiation of the species. Lastly there have been many changes in chromosome shape, and the occurrence of races

within a species which differ in the shape of one chromosome pair and of plants with heteromorphic chromosome pairs indicates that this is not a very uncommon occurrence in phylogeny.

On the whole it seems probable that a number of different mechanical processes affecting chromosome organization have played a part in the evolution of chromosomes in this genus. Processes such as fragmentation, union, inversion, deletion, translocation, and duplication have occurred in *Drosophila*. Painter and Muller (1929) and Muller and Painter (1929) have recently described changes in chromosome shape resulting from such processes actually induced by irradiation with X-rays. These studies are extremely significant in connection with problems of chromosomes and phylogeny, for such processes can account for changes in size and shape and increase and decrease in number of chromosomes. In particular they offer a possible explanation for the 6, 8, 12 portion of the *Crepis* chromosome series whose manner of origin from a supposed 10-chromosome ancestor presents the most difficult problem in the study of the evolution of chromosomes within the genus.

### SUMMARY AND CONCLUSIONS

The number and morphology of the somatic chromosomes of seventy species (including three which have been considered *Crepis* but will probably be assigned to other genera) were investigated in connection with a taxonomic study of the genus. The study of chromosome morphology has increased materially the value of the cytological findings in connection with classification.

Size differences, the occurrence of satellites, and shape as determined by spindle-fiber attachment were used to distinguish the various chromosomes.

The species discussed include twenty-seven whose chromosome numbers have not previously been counted and a number which have not been figured previously. A drawing showing a somatic metaphase which depicts as far as possible details of morphology is included for each species. The known series of chromosome numbers of Old World species is 6, 8, 10, 12, 14, 15, 16, and  $40\pm$ . That of the American species is 14, 22, 33, 44, 55?, and 88?. Without doubt these latter numbers are members of a polyploid series, as are some of the numbers in the Old World group. Others probably originated through interspecific hybridization. There is considerable evidence pointing

to 10 as the basic number. An alternative hypothesis is the assumption that 8 is the more primitive number in *Crepis*. No evidence exists at present to support this assumption.

In regard to size of chromosomes, there is a range of sizes, the extremes of which may be roughly expressed by the ratio 1:2. It should be noted, however, that the species having the smallest chromosomes (*senecioides*, *nana*, *elegans*, and *leontodontoides*) comprise only a small fraction of the entire number of *Crepis* species thus far studied. In comparison with these the chromosomes of the remaining species may be designated as medium, medium-large, and large, but within each of these categories there is considerable variation so that the entire list of species could be arranged in a nearly continuous series on the basis of chromosome size. Species representing the extremes in size differences occur in *Eucrepis*, while *Barkhausia* contains one species having chromosomes of the smallest size, and *Catonia* has several species with very large chromosomes. The variation in chromosome sizes, therefore, is not peculiar to any one subgenus; on the contrary it is distributed throughout the genus.

With regard to shape of the chromosomes, while there are many minor differences between individual species, there is a general similarity in all the species. Occurrence of satellites and position of constrictions are the most useful differences in shape. The occurrence of at least one pair of satellited chromosomes is practically constant throughout all the species. About one-third of the species have at least one pair of chromosomes with approximately median constrictions. In all other chromosomes the constrictions are located at some point nearer the proximal end of the chromosome.

A chart was drawn up to show the four major subdivisions of the genus and the chromosome numbers of the species discussed. This chart depicts the phylogenetic groups which were worked out by combining data on chromosome number and morphology with evidence from external morphology, consideration having been given to geographic distribution and evidence from interspecific hybridization. The chromosomes of the species in the various phylogenetic groups were discussed and their resemblances and differences noted.

In *Paleya*, considered the most primitive subgenus and supposed to contain or to have contained the progenitors of the other subgenera, only one species has been examined and it has 10 chromosomes. Probably further study would discover other chromosome numbers in this section. The subgenus *Barkhausia* contains species with 8, 10, and 16

chromosomes, and these same numbers and one 12-chromosome species are found in *Catonia*. *Eucrepis*, the largest subgenus, contains the greatest number of species and those whose connection with *Paleya* is least obvious. The numbers 6, 8, 10, 12, 14, 16, and 40 are found in the Old World species of the subgenus. With the exception of two closely related species, one of which also occurs in the Old World, the American species form a polyploid series with a base number of 11. These facts, together with the heterogeneity of *Eucrepis*, lead to the inference that this subgenus consists of a number of related phylogenetic lines and that the earlier connecting forms and the more primitive ancestors have all disappeared. Several of these diverse subgroups under *Eucrepis* are represented among the species thus far studied by individual species while others contain from two to six species which are obviously closely related both from their external morphology and their very similar chromosomes.

Two instances of heteromorphic chromosome pairs involving differences in point of spindle-fiber attachment were found (*C. pulchra* and *Pterotheca sancta*) and in the former species, in addition to the plant showing this condition, other plants with a pair of each of the different chromosomes were found. A difference in size of satellites on homologous chromosomes was noted in *C. foetida*.

In one case (*C. alpina* var. *syriaca*) variation in chromosome number from 10 to 13 was found and the variation appeared to involve one particular chromosome type.

The phylogenetic system proposed involves the assumption of several different kinds of chromosome changes. They include increase and decrease in chromosome number and increase and decrease in size and change in shape. Ways in which these changes may have come about are discussed and the most likely ones are pointed out.

In general, in each section of the genus, morphologically similar species have similar chromosomes and the writers are more firmly convinced by this investigation of the value of such a study of chromosomes in relation to taxonomy. Certainly there is a fairly close parallelism in *Crepis* between number and morphology of the chromosomes and phylogenetic relationship.

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**CYTOLOGICAL INVESTIGATIONS OF  
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**BY**

**LILLIAN HOLLINGSHEAD**

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# CYTOLOGICAL INVESTIGATIONS OF HYBRIDS AND HYBRID DERIVATIVES OF *CREPIS* *CAPILLARIS* AND *CREPIS* *TECTORUM*

BY  
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## INTRODUCTION

Concurrently with the investigation of a lethal factor which manifested itself in  $F_1$  hybrids of *Crepis capillaris* (L.) Wallr. and *C. tectorum* L. (Hollingshead, MS), a cytological investigation of the same material was undertaken. It included studies of the somatic and meiotic chromosomes of the parental species and of reciprocal hybrids, while the occurrence of several triploid hybrids ( $2n$  *capillaris*,  $n$  *tectorum*) provided material for a similar study of these and of some of their progeny.

Meiotic phenomena in interspecific hybrids in this genus have been described by Collins and Mann (1923), M. Nawaschin (1927), and Babcock and J. Clausen (1929). The hybrids described in this paper resemble those investigated by Babcock and Clausen, but differ from the comparable ones described by Collins and Mann and by Nawaschin in exhibiting a variable number of pairs in meiosis. Unpublished investigations on the number of bivalents and univalents in various other hybrids, by Dr. Margaret Mann Lesley, Miss Priscilla Avery, and the writer, have shown that this phenomenon is common in many interspecific hybrids in the genus.

## ACKNOWLEDGMENTS

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## MATERIALS AND METHODS

The *capillaris* strain X which was used in most of the hybrids is of unknown origin, having descended from a stray plant picked up in the greenhouse. The other strains were kindly supplied by Dr. J. L. Collins, and are of various origins. Two *tectorum* strains (1498, from Copenhagen Botanical Garden, and 1648, from Tomsk, Siberia) were involved in the hybrids examined. The 1498 strain appeared to be morphologically rather constant and had descended from one plant (Hollingshead, MS). Plants of the 1648 strain, from original wild seed, varied in leaf shape and time of maturity. Plate 1 shows typical *capillaris*, *tectorum*, and  $F_1$  hybrid plants at maturity.

Sections of root tips fixed in chromacetic formalin as described by Hollingshead and Babcock (1929), and stained with Heidenhain's iron-haematoxylin were used for somatic chromosome studies. Meiotic phases were obtained from buds fixed in Carnoy's fluid, washed in absolute or 95 per cent alcohol, run through 80 per cent alcohol into 70 per cent, there to remain until examined. Material so fixed usually remained in good condition for at least a year. Considerable shrinkage was occasionally noted but on the whole the fixation was good. To make mounts from such material the florets were dissected out on a slide, crushed in a drop of aceto-carmin, the debris removed, a coverslip applied, and all excess fluid absorbed so that only a thin film remained and the coverslip held the loose pollen-mother-cells (PMC's) in place. The counts given later in the paper, each from one slide, will give some idea of the number of countable PMC's obtained from one bud in favorable material. In most cases the PMC's remained in rows of a few to many cells and these were usually at the same stage of development. Pollen grains were mounted in acetocarmine and were counted "good" if the cytoplasm was well stained after twenty-four hours.

All examinations were made with a Bausch and Lomb 90 $\times$  apochromatic objective and Zeiss compensating oculars. The somatic chromosomes were drawn at a magnification of 3750, the meiotic at 2450, and the tetrads at 1250 and all were reduced one-third in reproduction.



## SOMATIC CHROMOSOMES OF PARENTS AND HYBRIDS

*Crepis capillaris*, as shown by Taylor (1925, 1926) and M. Nawaschin (1925b), has six somatic chromosomes which are readily distinguished as three pairs by their distinctive morphology. Nawaschin (1925b) has figured those of *C. tectorum* in which there are four pairs of chromosomes, each different. Somatic metaphase plates of these two species showing the same details of morphology as Taylor and Nawaschin have shown are given in figure A. For the sake of

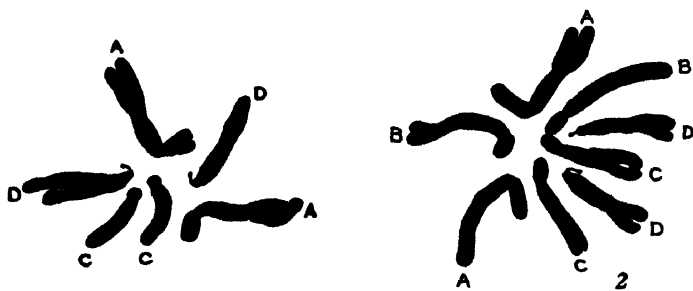


Fig. A. Somatic metaphases of (1) *C. capillaris*, (2) *C. tectorum*.

uniformity with Nawaschin's system of designation, the three chromosomes of *capillaris* will be designated A, C, and D, and those of *tectorum* A, B, C, and D (fig. A). The writer has no intention of signifying homology by these letters.

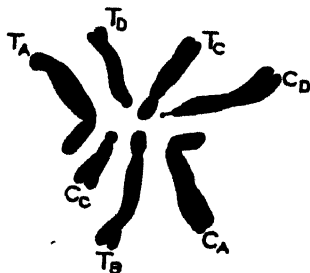


Fig. B. Somatic metaphase of  $F_1$  *C. capillaris-tectorum*.

Nawaschin (1927) has figured the somatic chromosomes of the  $F_1$  *capillaris-tectorum* hybrid and the writer's investigations confirm his observation that the *tectorum* D-chromosome in the hybrid is modified so that the satellite is visible no longer and the head is slightly enlarged (fig. B). The chromosomes of reciprocal hybrids are alike in this respect.

Measurements of chromosome length seem to indicate that this may not be the only chromosome change which accompanies hybridization. Measurements were made on all the chromosomes in each species and on *capillaris* chromosomes A and C and *tectorum* chromosomes A, B, and C, in reciprocal hybrids. Ten representatives of each chromosome from the protoderm or the row of cells immediately below it, lying quite or, in a few cases, almost horizontally, were drawn at the same magnification, projected by a lantern, and the figures so obtained were traced and the lengths measured by means of an opisometer run along the median line and back along a measured straight edge. Each chromosome was measured twice, or until two measurements agreed. The instrument, or the manipulation of it, gave measurements which varied rarely more than 0.2 cm. to a chromosome. All material from which measurements were made was fixed in the same fixative and treated in the same way.

Table 1 gives the individual lengths and mean lengths obtained for chromosomes A and C of *capillaris* and A, B, and C of *tectorum* in the parental species and in reciprocal hybrids. The D-chromosomes of each species were not measured in the hybrids and so are not included in the table but the measurements on the species confirmed the opinion derived from observation that the D-chromosome of *capillaris* is slightly longer than that of *tectorum*.

Three different strains and a number of different plants were involved in the three sets of measurements of *capillaris* chromosomes and it might be that if there is any constant difference in the length of any of the *capillaris* chromosomes between the hybrids and the parental species, it might be the result of this condition. All the *tectorum* measurements were from one strain but involved several plants. The data show that considerable variation occurs in the lengths of the same chromosome in different cells and that the chromosomes from the protoderm (bracketed together at the top of each set of measurements) are on the whole somewhat longer than those in the cells beneath. There are in each set of measurements representatives from each of the two rows of cells from which chromosomes were measured. Table 2 shows the differences in mean lengths obtained for the various chromosomes between the parental species and reciprocal hybrids, the probable errors of the differences, and the relation of the differences to their probable errors.

Most of the chromosomes showed no significant difference in mean length in parental species and in hybrids. The A-chromosome of

TABLE 1

LENGTHS OF TEN REPRESENTATIVES OF FIVE DIFFERENT CHROMOSOMES OF  
*C. capillaris* AND *C. tectorum* FROM THE ROOT TIPS OF THE  
 PARENTAL SPECIES AND RECIPROCAL HYBRIDS

|  | <i>capillaris</i> |       | <i>tectorum</i> |       |       |
|--|-------------------|-------|-----------------|-------|-------|
|  | A                 | C     | A               | B     | C     |
| Parental species                           | 18.0              | 8.5   | 19.6            | 15.1  | 14.8  |
|  | 18.0              | 8.7   | 20.7            | 17.6  | 15.1  |
|  | 18.3              | 8.8   | 19.3            | 14.3  | 15.5  |
|  | 21.0              | 11.0  | 19.4            | 17.8  | 14.1  |
|  | 20.2              | 9.7   | 19.9            | 18.6  | 14.0  |
|  | 17.2              | 9.3   | 18.7            | 17.4  | 13.2  |
|  | 16.6              | 9.2   | 17.3            | 16.1  | 14.2  |
|  | 16.8              | 8.5   | 17.0            | 15.5  | 14.8  |
|  | 18.0              | 8.9   | 18.5            | 16.8  | 14.2  |
|  | 17.5              | 8.9   | 19.3            | 15.1  | 13.9  |
| Mean                                       | 18.16             | 9.15  | 18.97           | 16.43 | 14.38 |
| <i>tectorum</i> ♀ ×<br><i>capillaris</i> ♂ | 18.2              | 10.8  | 17.4            | 18.0  | 13.5  |
|  | 20.1              | 9.7   | 17.3            | 17.0  | 12.8  |
|  | 17.0              | 11.0  | 21.1            | 16.4  | 13.2  |
|  | 16.3              | 10.1  | 19.9            | 18.0  | 13.1  |
|  | 17.8              | 10.4  | 18.8            | 17.5  | 13.4  |
|  | 16.0              | 8.9   | 17.8            | 19.3  | 14.2  |
|  | 19.2              | 9.8   | 19.0            | 17.1  | 12.7  |
|  | 17.5              | 10.5  | 18.2            | 16.7  | 13.3  |
|  | 17.6              | 10.7  | 15.8            | 16.8  | 11.5  |
|  | 19.6              | 9.3   | 18.8            | 16.2  | 11.8  |
| Mean . . . . .                             | 17.92             | 10.12 | 18.41           | 17.3  | 12.95 |
| <i>capillaris</i> ♀ ×<br><i>tectorum</i> ♂ | 19.0              | 9.3   | 18.3            | 18.8  | 14.2  |
|  | 18.5              | 10.2  | 17.7            | 16.4  | 13.8  |
|  | 19.2              | 10.5  | 17.2            | 16.1  | 13.7  |
|  | 20.1              | 9.7   | 17.5            | 15.8  | 11.9  |
|  | 17.3              | 10.0  | 18.4            | 15.7  | 13.7  |
|  | 16.0              | 8.8   | 18.9            | 16.0  | 11.8  |
|  | 20.3              | 9.2   | 19.5            | 16.8  | 13.1  |
|  | 16.9              | 9.7   | 16.3            | 16.6  | 13.4  |
|  | 18.9              | 9.9   | 15.3            | 16.6  | 11.8  |
|  | 17.0              | 10.5  | 16.7            | 15.4  | 12.7  |
| Mean.....                                  | 18.32             | 9.78  | 17.58           | 16.42 | 13.01 |

TABLE 2

DIFFERENCES IN MEAN LENGTHS OF FIVE CHROMOSOMES IN THE PARENTAL SPECIES  
AND RECIPROCAL HYBRIDS

| Chromosome          | Mean length<br>in parents | Hybrid              | Mean length<br>in hybrid | Parental<br>-hybrid<br>mean | Probable<br>error of<br>difference | Diff.<br>Ed |
|---------------------|---------------------------|---------------------|--------------------------|-----------------------------|------------------------------------|-------------|
| <i>capillaris</i> A | $18.16 \pm 0.286$         | <i>capillaris</i> ♀ | $17.92 \pm 0.272$        | +0.24                       | $\pm 0.395$                        | 0.6         |
|                     |                           | <i>tectorum</i> ♀   | $18.32 \pm 0.293$        | +0.16                       | $\pm 0.409$                        | 0.4         |
| <i>capillaris</i> C | $9.15 \pm 0.710$          | <i>capillaris</i> ♀ | $9.78 \pm 0.530$         | +0.63                       | $\pm 0.189$                        | 3.3         |
|                     |                           | <i>tectorum</i> ♀   | $10.12 \pm 0.138$        | +0.97                       | $\pm 0.205$                        | 4.7         |
| <i>tectorum</i> A   | $18.97 \pm 0.229$         | <i>tectorum</i> ♀   | $18.41 \pm 0.298$        | -0.56                       | $\pm 0.376$                        | 1.5         |
|                     |                           | <i>capillaris</i> ♀ | $17.58 \pm 0.255$        | -1.39                       | $\pm 0.344$                        | 4.0         |
| <i>tectorum</i> B   | $16.43 \pm 0.286$         | <i>tectorum</i> ♀   | $17.30 \pm 0.188$        | +0.87                       | $\pm 0.343$                        | 2.5         |
|                     |                           | <i>capillaris</i> ♀ | $16.42 \pm 0.191$        | -0.01                       | $\pm 0.354$                        | 0.03        |
| <i>tectorum</i> C   | $14.38 \pm 0.135$         | <i>tectorum</i> ♀   | $12.95 \pm 0.162$        | -1.43                       | $\pm 0.211$                        | 6.7         |
|                     |                           | <i>capillaris</i> ♀ | $13.01 \pm 0.183$        | -1.37                       | $\pm 0.228$                        | 6.0         |

*tectorum* had a shorter mean length in both hybrids, 1.5 and 4 times the probable error respectively, which in view of the small number of measurements cannot be said to be very significant. An increase in length in the C-chromosome of *capillaris*, 4.7 and 3.3 times the probable error in reciprocal hybrids, may be significant and might be attributed to hybridization if the same strain had been used in all measurements. The data on *tectorum* C showing a difference of over 6 times the probable error in each case would seem to indicate clearly that this chromosome was constantly shorter in the hybrids examined than in the plants of the species examined.

The data can be considered only as of a preliminary nature, but they suggest that certain chromosomes in a hybrid may be constantly modified in size as well as in form. Where the difference is at all great the modification is in the same direction in reciprocal hybrids and would suggest that the change was a result of a mutual reaction between the chromosomes of the two hybrid complexes. Yet it is evident that all the chromosomes of a complex were not affected in the same way. It would appear worth while to investigate this and other suitable material more extensively in this connection.

## MEIOSIS IN THE PARENTAL SPECIES

Stages prior to diaphase (terminology, Belling, 1928) have not been studied in either parents or hybrids. With the exceptions to be noted, meiosis proceeds similarly in the two parental species and the descriptions apply equally to both. Middle diaphase figures usually show chromosome pairs elongated and more or less loosely associated, as depicted for *capillaris* in figure C, 1. One of the pairs seems often to lie with an end on the nucleolus and in a number of cases in *capillaris* this pair has been determined as the middle-sized one. Since it is this pair which bears the satellites in somatic cells, this agrees

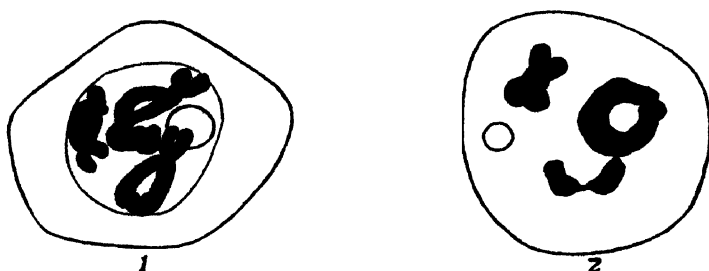


Fig. C. (1) Middle and (2) late diaphase of *C. capillaris*.

with the findings of those who have reported satellites associated with the nucleolus (cf. Kuhn, 1928). Late diaphase, a stage evidently passed through rather quickly, for only occasionally does a slide show many such figures, is particularly favorable for showing size differences in chromosome pairs (*capillaris*, fig. C, 2). The shapes of the bivalent chromosomes are not constant.

First metaphase (I-M) plates of the two species with three and four bivalents, respectively, are given in figure D, 1 and 2. Again the shapes of the bivalents are not constant. In *capillaris* it is usually possible to distinguish at least the smallest pair by its size, but this stage is less favorable than the one just described for determining size differences. All the first late anaphase (I-A) chromosomes show a longitudinal split similar to that figured later for hybrids. At second metaphase (II-M) and anaphase (II-A) the chromosomes resemble those in somatic divisions (fig. E, 1 and 2) and here they can often be distinguished from one another.

With the exception of an occasional case of non-conjunction (3 out of 47 PMC's in one slide showed this condition), meiosis in the

*tectorum* plants examined proceeded normally and regular tetrads were formed. So much cannot be said for *capillaris*, for in the X-strain of this species, which was the one most studied, meiotic irregularities occurred frequently. Three plants of this strain on which I-M counts were made showed higher percentages of irregularities in meiosis and tetrads than did any of the *tectorum* plants examined, but very noticeable differences in the amount of irregularities were evident between the various plants. The most frequent form of

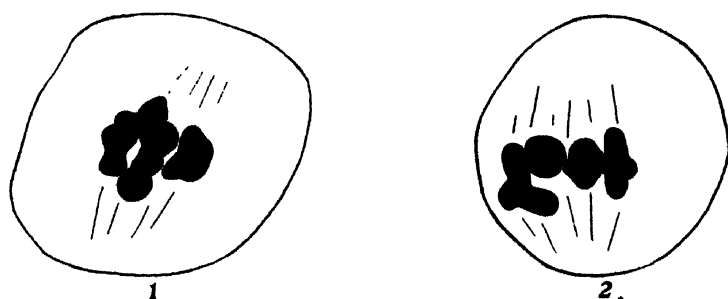


Fig. D. Side views of meiotic metaphases of (1) *C. capillaris*, (2) *C. tectorum*.

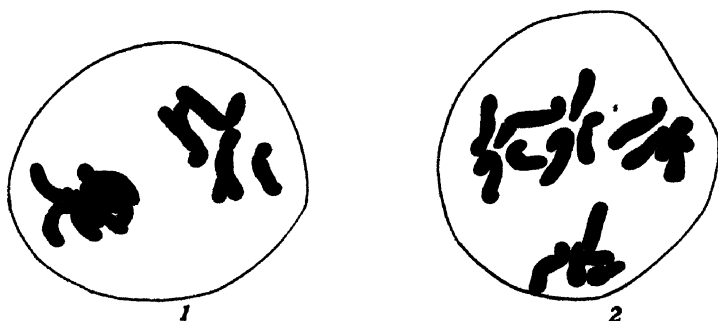


Fig. E. Second meiotic metaphase and anaphase of *C. tectorum*.

irregularity was non-conjunction of the members of one pair of chromosomes but occasionally four chromosomes were unpaired. Non-conjunction occurred at diaphase (fig. F, 1 and 2) as well as at metaphase (fig. G, 1 and 2). When one pair of chromosomes failed to conjugate, it was apparently not always the same pair (fig. F, 1 and 2). Abnormal elongation of bivalents and fragmentation at I-M occurred frequently in some slides (fig. H, 1 and 2).

Table 3 shows the amount of non-conjunction observed in several representative plants of this species, each count having been made from a single bud. Though admittedly inadequate, the results may indicate that strains other than X are more nearly regular in meiotic phenomena, and this is upheld by pollen counts (below). Plants

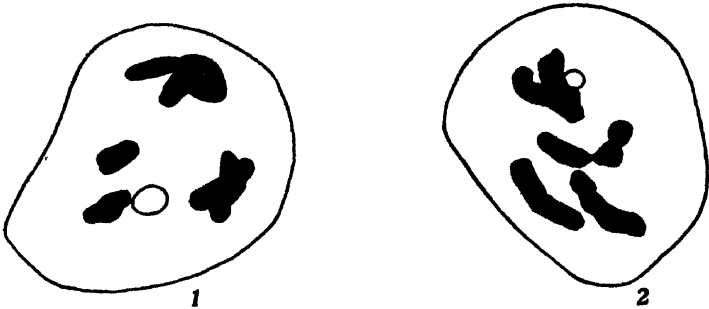


Fig. F. Late diaphases of *C. capillaris* showing non-conjunction in different chromosome pairs.

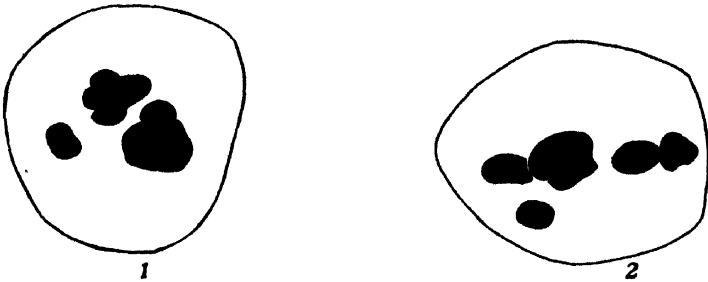


Fig. G. Meiotic metaphases of *C. capillaris* showing non-conjunction of (1) one-chromosome and (2) two-chromosome pairs.

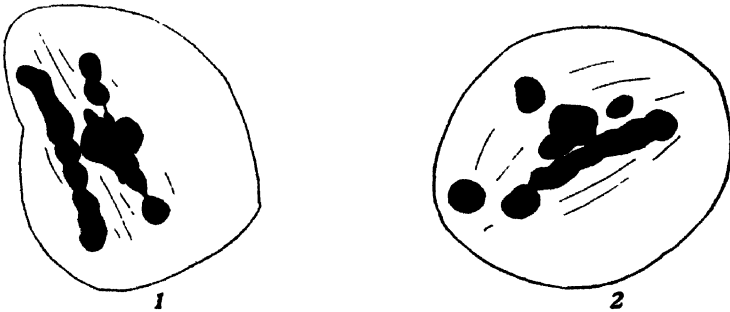


Fig. H. First meiotic metaphases of *C. capillaris* showing elongation of bivalents and fragmentation.

TABLE 3

FIRST METAPHASE COUNTS FROM SINGLE BUDS OF PLANTS OF *C. capillaris* SHOWING THE AMOUNT OF NON-CONJUNCTION OBSERVED

| Plant    |            | 3"  | 2'+2' | 1'+4' |
|----------|------------|-----|-------|-------|
| X-strain | 27H. 21-4  | 63  | 9     | .. .  |
|          | 28H. A-7   | 84  | 62    | 4     |
|          | 29. 169-3  | 20  | 78    | .. .  |
|          | 29. 169-3  | 45  | 89    | 1     |
|          | 27. 12-23  | 153 | 1     | ..... |
|          | 28. 142-11 | 66  | 4     | ..... |

29.169-3 of the X-strain and 28.142-11 of another strain were flowering in winter when *capillaris* does not thrive. The former showed a great number of irregularities while the latter was nearly normal in spite of the fact that the material was secured when the plant had almost completed its life and was beginning to die—circumstances which have been found to be associated with meiotic irregularities (Hakansson, 1926). This indicates that high percentage of irregularities cannot be ascribed to unsuitable environment and this is further upheld by the fact that the other plants of the X-strain flowered in the summer under essentially similar conditions and showed equally wide variation in amount of irregularity. Root tips of 29.169-3, which was the most irregular of any plant studied at all extensively, showed a normal somatic chromosome complex.

Tetrad studies showed different amounts of irregularities in the shape of micronuclei, microcytes, supernumerary cells, and diads in different plants of the X-strain, but no irregularities were observed in two plants of another strain (27.12-23, which showed almost regular I-M, table 3, and a sister plant not examined at I-M). Pollen studies gave results to be expected from the meiotic phenomena. While *tectorum* plants gave almost always 95 per cent or more good pollen, the X-strain of *capillaris* gave generally higher percentages of bad pollen than plants of *tectorum* and other strains of the same species, the percentage varying greatly from plant to plant. Table 4 gives various pollen counts from single heads of 12 plants of the X-strain and 3 plants of other strains. The second to the eleventh plants are selfed progeny of the first plant, the twelfth is a third-generation plant from the same source, while the other three represent other strains. All counts were made from healthy plants grown in the summer except 29.169-3 and 28.142-11, which flowered in the winter.

With the exception of the third and fifth plants, counts made from different heads of the same plant on the same or different days showed little difference in percentage of good pollen, and even with them the difference was not very great. The various sister plants of the X-strain showed wide differences in the amount of good pollen, which in several cases was known to have been associated with corresponding differences in the amount of meiotic irregularities. Thus 27.12-23 (no. 14 in table 4) was almost completely regular at I-M (table 3) and had scarcely any bad pollen, plant 27H.21-4 (no. 6) had a few more I-M irregularities and had a higher percentage of



TABLE 4

POLLEN COUNTS FROM VARIOUS FLOWERS OF FIFTEEN PLANTS OF *C. capillaris*,  
WHERE BRACKETED TOGETHER THE COUNTS WERE MADE ON THE SAME DAY

| Plant         | Number of grains |              | Bad<br><br>per cent |
|---------------|------------------|--------------|---------------------|
|               | Good             | Bad          |                     |
| 1 26. X-2     | 596              | 36           | 5.7                 |
| 2 27H. 8-2    | { 418            | 11           | 2.5                 |
|               | { 365            | 17           | 4.4                 |
| 3 27H. 8-4    | { 359            | 47           | 11.6                |
|               | { 366            | 47           | 11.4                |
|               | { 403            | 110          | 21.4                |
|               | { 566            | 51           | 8.3                 |
|               | { 420            | 39           | 8.5                 |
|               | { 397            | 38           | 9.6                 |
| 4 27H. 8-12   | { 6              | 237          | 97.5                |
|               | { 17             | 234          | 93.2                |
|               | { 23             | 209          | 90.1                |
|               | { 25             | 226          | 90.0                |
| 5 27H. 8-13   | { 59             | 309          | 74.3                |
|               | { 49             | 410          | 89.3                |
| 6 27H 21-4    | 672              | 114          | 14.5                |
| 7 27X-1       | { 434            | 63           | 12.7                |
|               | { 606            | 62           | 9.3                 |
| 8 27X-3       | { 234            | 375          | 61.6                |
|               | { 94             | 170          | 64.4                |
| 9 27X-12      | 376              | 137          | 26.7                |
| 10 27X-13     | { 31             | 211          | 87.2                |
|               | { 34             | 704          | 95.4                |
| 11 27X-14     | 567              | 144          | 20.2                |
| 12 29 169-3   | 197              | 446          | 69.4                |
| 13 27. 1817-5 | almost all       | scarcely any | 1—                  |
| 14 27. 12-23  | almost all       | scarcely any | 1—                  |
| 15 28. 142-10 | 488              | 18           | 3.5                 |

bad pollen, and 29.169-3 (no. 12) was highly irregular at I-M and had a very high percentage of bad pollen. Plant 27H.8-12 (no. 4), which had the highest percentage of bad pollen, had the most irregular tetrads of any plant examined; an examination of a single slide showed many irregular I-M plates and only a few with the normal three bivalent chromosomes. Plants which were very irregular were completely, or almost completely, sterile.

This probably indicates that gametes with abnormal chromosome complexes resulting from irregular meiosis usually fail to develop and that generally only normal gametes function. During the investigation more than 2500 interspecific hybrids involving various

plants of the X-strain of *capillaris* were obtained and, with the following exceptions, *capillaris* plants appeared to have given normal hybrids with *tectorum*. The exceptions were: (1) a number of hybrids contained a diploid set of *capillaris* and a haploid set of *tectorum*, and (2) one hybrid examined contained the haploid complexes of the parental species and a single extra chromosome of *capillaris*. The first would indicate that there is a considerable number of female gametes with the somatic chromosome complex formed in *capillaris* and that they function, as had already been supposed from the occurrence of polyploids and triploid hybrids (Nawaschin, 1925b, 1927). It was noted above that diads were seen occasionally and large pollen grains were observed rather often in some slides, indicating perhaps that somatic male gametes were also formed, although non-reduction was not actually observed. The second exception shows that female gametes with an extra chromosome resulting from meiotic irregularity may function occasionally, which has already been supposed from the occurrence of trisomics in this species (Nawaschin, 1926).

### MEIOSIS IN CAPILLARIS-TECTORUM $F_1$ HYBRIDS

The outstanding characteristics of the meiosis of these  $F_1$  hybrids are a variable number of bivalents and a variable behavior of univalents. Two groups of hybrids involving two different strains of *tectorum* were studied in this connection and I-M counts of the number of bivalents and univalents were made on seven plants of the first group and three plants of the second. The results are given in table 5 which shows the frequencies of all the possible combinations of bivalents and univalents observed in the various plants. Each line represents the results obtained from a single slide made from a single bud and includes all the cells in which the bivalents and univalents could be counted and distinguished from each other. The two middle columns contain the combinations hardest to distinguish and the actual proportion of these cells may have been somewhat higher than the counts represent.

A striking difference in distribution between the first and second groups was observed as soon as plants of the second class were examined. Unfortunately material had been preserved from only three plants of the second group since no such difference had been anticipated. Each group included hybrids grown in two different seasons.

TABLE 5

THE FREQUENCIES OF THE VARIOUS COMBINATIONS OF BIVALENTS AND UNIVALENTS  
IN *capillaris-tectorum* F<sub>1</sub> HYBRIDS INVOLVING TWO DIFFERENT  
*tectorum* STRAINS

| Parental<br><i>tectorum</i><br>strain | Hybrid     | 3'+1' | 2'+3' | 1'+5' | 7'  |
|---------------------------------------|------------|-------|-------|-------|-----|
| 1498                                  | 27 H 17-1  | 37    | 37    | 7     | ... |
|                                       | 27 H 17-2  | 34    | 49    | 15    | 3   |
|                                       | 27 H 17-4  | 19    | 15    | 6     | ... |
|                                       | 27 H 20-1  | 12    | 7     | 1     | .   |
|                                       | 27 H 15-6  | 31    | 14    | 2     | ... |
|                                       | 27 H 15-2  | 12    | 3     | 3     | .   |
|                                       | 28 H 17-1  | 47    | 16    | .     | ... |
|                                       |            | 52    | 17    | 3     | ... |
|                                       | Total      | 244   | 158   | 37    | 3   |
| 1648                                  | 27 H 19-5  | 32    | 44    | 44    | 45  |
|                                       | 27 H 12-1  | 12    | 21    | 16    | 20  |
|                                       | 28 H 128-4 | 16    | 39    | 31    | 38  |
|                                       |            | 45    | 66    | 53    | 54  |
|                                       | Total      | 105   | 170   | 144   | 157 |

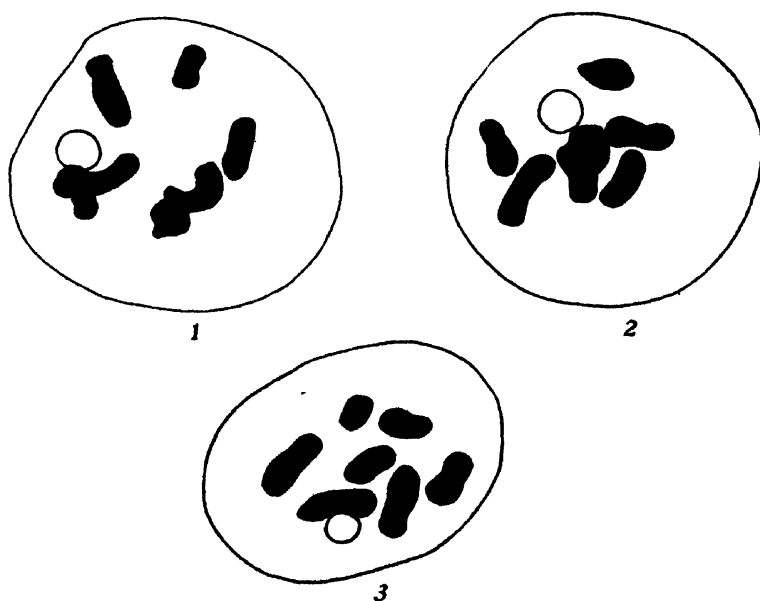


Fig. I. *C. capillaris-tectorum* F<sub>1</sub>, late diaphase, showing respectively (1) two, (2) one, and (3) no bivalents.

The plants of the group involving the 1498 strain usually showed three bivalents and one univalent, fewer cases of two bivalents and three univalents, and occasionally one bivalent and five univalents, and rarely or never seven univalents. Some variation in the proportion of these classes occurred from plant to plant but the type was similar in all. The three hybrids involving the 1648 strain showed a very different distribution. In these plants each possible combination was represented in approximately equal proportions, the combination of three bivalents and one univalent, which was most

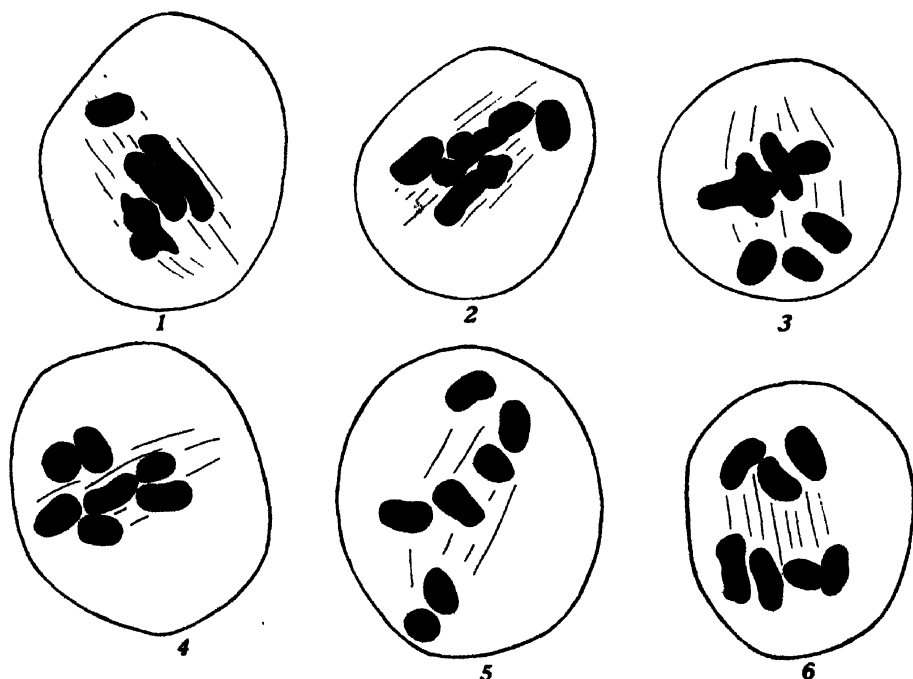


Fig. J. *C. capillaris-tectorum* F<sub>1</sub>, first meiotic metaphase, (1) three bivalents, (2) two bivalents, (3) one bivalent, (4-6) no bivalents.

frequent in the first group, being least frequent here. There could be no doubt that for the plants examined a very different type of distribution of possible combinations characterized the two groups.

Three PMC's at late diaphase representing the second, third, and fourth combinations are shown in figure I. Size differences can be seen but it is impossible to distinguish the various chromosomes. Cells at I-M showing all possible combinations of bivalents and univalents are shown in figure J. The bivalents resemble those in the parental species and are not constant in shape. The univalents usually lie away from the metaphase plate but one or more may lie beside the bivalents (fig. J, 2, 3). The cells containing seven unpaired chromo-

somes are especially striking. The univalents appear to be scattered throughout the cytoplasm and do not form a typical metaphase plate. Their arrangements frequently simulate anaphases (fig. J, 5, 6), but that these are not anaphases is shown by their association with cells at metaphase and by the fact that none yet show the split which is characteristic of anaphase chromosomes. Slightly later such figures

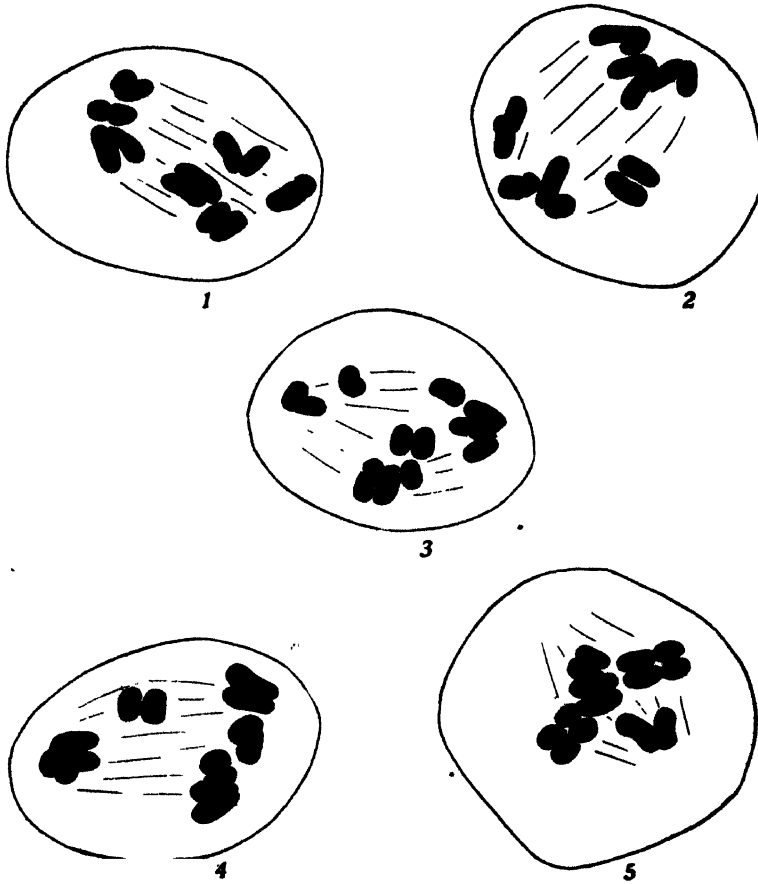


Fig. K. *C. capillaris-tectorum* F<sub>1</sub>, first meiotic anaphases showing both segregation and division of univalents.

as those shown in figure J, 5 and 6 will pass over into anaphase without any major change in the relative positions of the chromosomes. Occasionally a very unequal distribution of chromosomes, such as six at one pole and one at the other, was seen, but no certain case of seven univalents at one pole was observed. Figure J, 4 shows seven in a group nearer one pole than the other but the ones near the middle may move toward the other pole in the following anaphase.

The kind of anaphase is apparently determined by the number of univalents and the positions they assumed at metaphase. Various

I-A arrangements are shown in figure K. If the univalents have lain on or near the plate at I-M they apparently divide, the halves going to either pole (fig. K, 2-5). If they were not on the plate they move toward the nearest pole with the dissociated bivalent partners (fig. K, 1, 4). Figure K, 1, shows a cell in which no univalents have divided and there are three split chromosomes at one end and four at the other. Figure K-2 shows one univalent dividing on the plate with three split chromosomes at each pole. The other anaphase figures

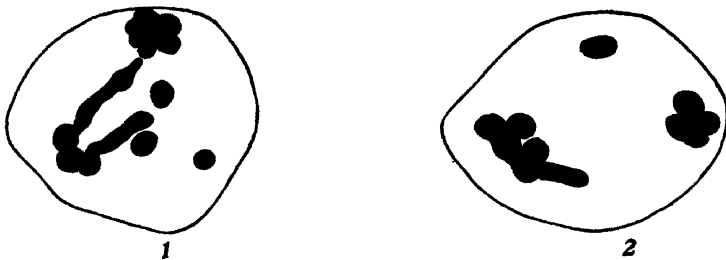


Fig. L. *C. capillaris-tectorum* F<sub>1</sub>, first meiotic anaphases showing "unclean" division, fragmentation, and lagging chromosomes.

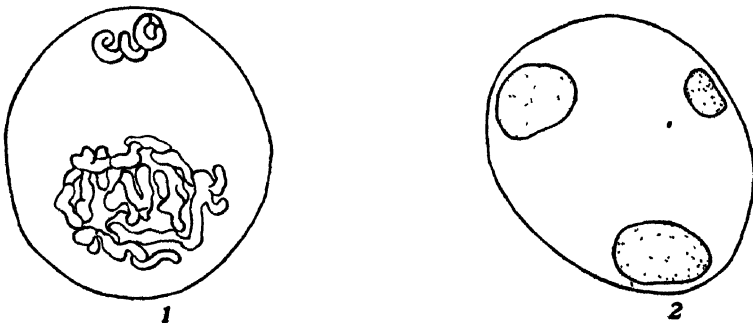


Fig. M. *C. capillaris-tectorum* F<sub>1</sub>, (1) second meiotic prophase with very unequal nuclei, (2) micronucleus at interphase.

doubtless represent cases in which less than three bivalents had been formed. Figure K, 5 shows a case of five splitting univalents on the plate, the highest number which was seen dividing at this stage. It may have arisen from a cell with one bivalent or from a cell in which all the chromosomes were unpaired and five of them lay on or near the plate. The former seems more likely.

An "unclean" separation of partners of bivalents, which has been frequently described for other hybrids, and extreme attenuation of anaphase chromosomes sometimes resulting in fragmentation were seen occasionally in some slides and rather frequently in others. Figure L, 1 is a drawing of a I-T figure showing "unclean" separation and fragmentation. In figure L, 2 a chromatin body which may

be either a chromosome or a fragment has been left in the cytoplasm and such a condition is not uncommon. Micronuclei are seen frequently in interphase (fig. M, 2). No case of a single nucleus at interphase was seen but instances of nuclei of very different size were occasionally observed. Figure M, 1 shows a case in which one nucleus in second prophase apparently consists of a single chromosome.

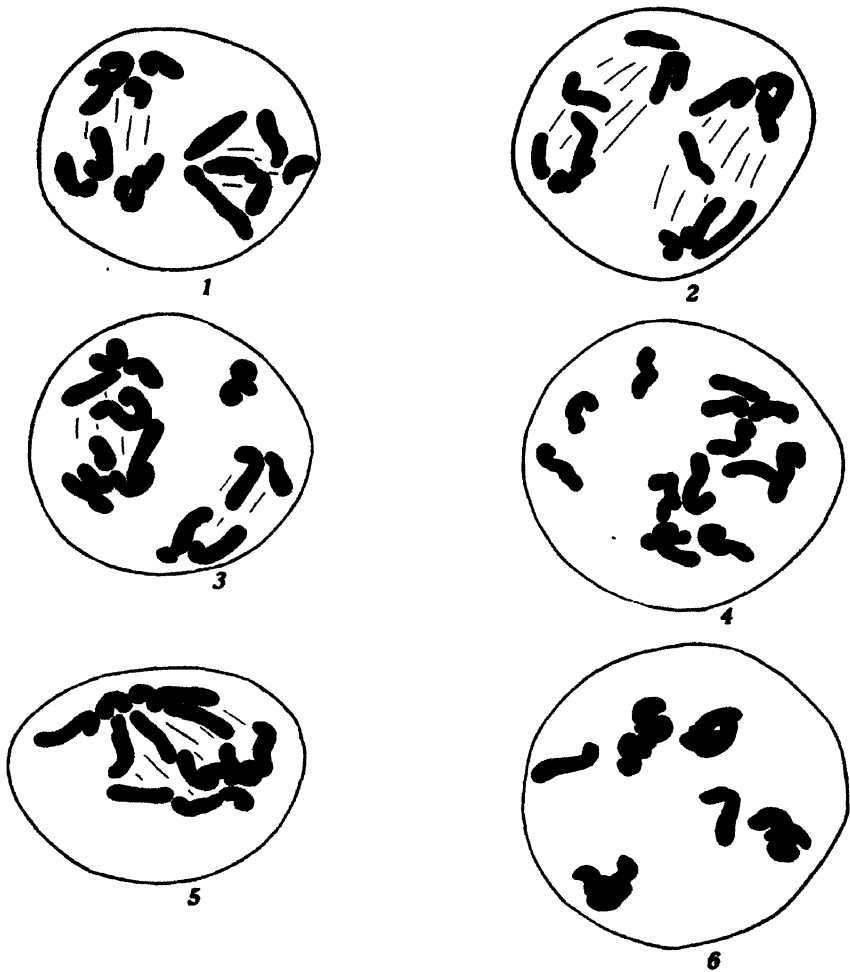


Fig. N. *C. capillaris-tectorum* F<sub>1</sub>, (1-5) second meiotic anaphases showing both division and segregation of univalents, (6) second telophase showing chromosome elimination.

Second divisions present a regular or irregular appearance depending on the nature of the preceding division. A first division in which the univalent chromosome or chromosomes did not divide but were included in the interphase nuclei apparently is followed by a regular second division in which all the chromosomes divide (fig. N, 1). A first division in which the univalent chromosome or chromo-

somes divided and the halves were incorporated in the interphase nuclei gives rise presumably to an anaphase with the univalent halves lagging on the spindle (fig. N, 2) and finally passing undivided to the poles. Figure N, 3 and 4, probably arose from interphases which contained micronuclei. In figure N, 3 at least one univalent half is passing undivided to a pole along with the halves of the bivalent partners. That the univalent chromosomes divide at either division is evident from the figures. However, a study of thirty clear second anaphases showed a total of fourteen chromosomes in every cell, indicating that the univalents divide only once during meiosis. An excessive drawing out of chromosomes at II-A similar to that described for I-A occurs occasionally, and fragmentation was evident in a few cases.

In the cell shown in figure N, 4 there was only one II-M plate and it contained all but three chromosomes; it presumably arose from an interphase with one large nucleus and one or more micronuclei. Figure N, 5 depicts a clear instance of a process which would give rise to somatic gametes. The seven divided chromosomes are of the second-division type and the cell was in a row of cells showing second-division figures. It could have arisen from a single interphase nucleus which resulted from a segregation of seven univalent chromosomes to one pole. Although such an occurrence was not seen, it has been pointed out above that very unequal segregations were observed and it is likely that all the chromosomes will occasionally pass to one pole. On the other hand, it is possible that a restitution nucleus, such as that described by Rosenberg (1926), may have been formed following the first division, although no evidence of any such process was seen.

It is evident from second-anaphase figures that nuclei with different numbers of chromosomes will sometimes occur and this is borne out by second-telophase figures where nuclei of various sizes may be seen. Figure N, 6 shows two pairs of nuclei of different sizes containing several chromosomes each and two chromosomes lying in the cytoplasm which would probably have given rise to micronuclei. The tetrads (fig. O), particularly from the hybrids involving the 1648 strain of *tectorum*, show frequent irregularities. Micronuclei and microcytes of various sizes are common and diads with and without micronuclei and microcytes are often found. It is easy to picture how some of the tetrads shown may have arisen from second anaphases, for instance figure O, 1 from such a cell as shown in figure N, 1; figure O, 7, from figure N, 4, and figure O, 8, from figure N, 5.



Some variation occurred in the percentage of well stained pollen obtained from different plants, but it was low in all cases, usually less than 1 per cent. Two  $F_1$  plants had a considerably higher percentage (15 per cent and 21 per cent). One of these proved to be a triploid hybrid (below), the other was not examined cytologically. Considerable variation in pollen size occurred and large well stained grains, presumably arising through non-reduction, were found quite frequently.

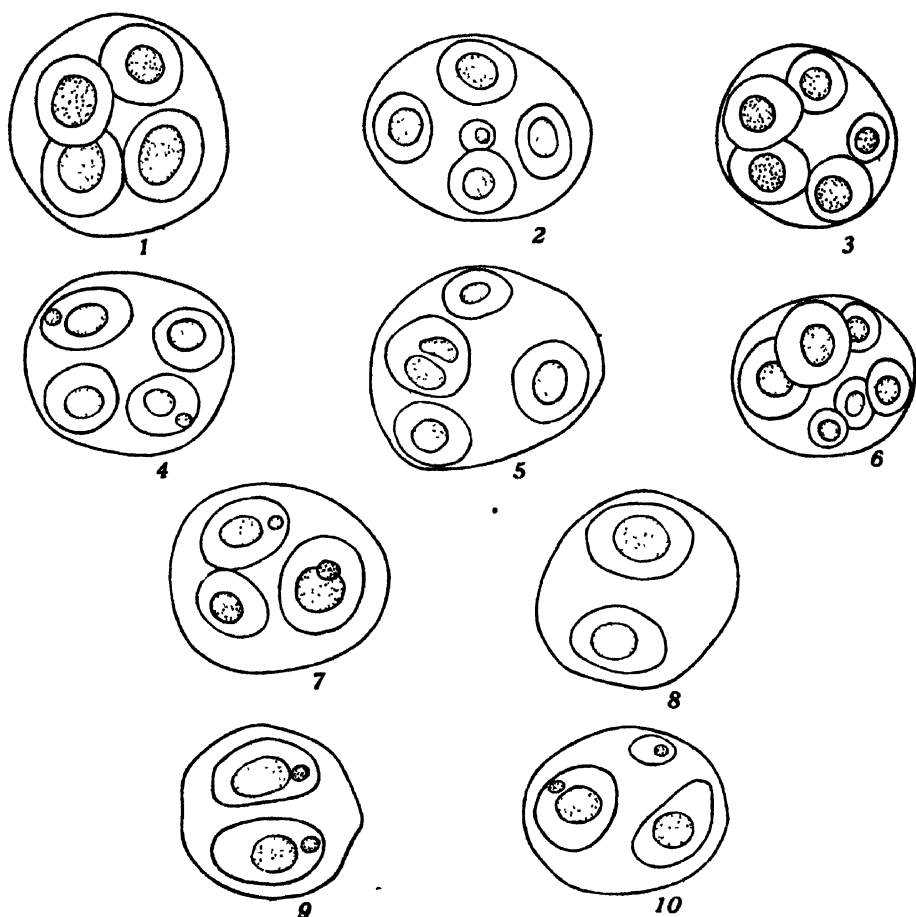


Fig. 10. Tetrads of *C. capillaris-tectorum*  $F_1$ .

The meiotic behavior of this hybrid differs from that described by Collins and Mann (1923) for *Crepis setosa*-*C. capillaris* and from that described by Nawaschin (1927) for *C. capillaris*-*C. aspera*  $F_1$  hybrids in the fact that bivalents are formed. Each of these hybrids, like the *capillaris-tectorum* hybrids described above, is a hybrid of *capillaris* with a four-chromosome species.

The *capillaris-setosa* hybrid (Collins and Mann, 1923) was among the first examined in the genus and has been widely quoted in the literature as an example of complete lack of chromosome pairing. In later (unpublished) investigations, however, Mann Lesley found a variable number of bivalents in this hybrid. The later material involved different strains of both species. It was shown above that *capillaris-tectorum* hybrids involving different strains of *tectorum* exhibited a marked difference in the frequency of cells with the various possible combinations of univalents and bivalents. In one group of hybrids the cells with all the chromosomes unpaired were very frequent and especially striking. In the other group they were extremely rare and the occurrence of bivalents was the rule. It is probable that such a phenomenon may explain the difference between Mann Lesley's earlier and later observations. Possibly the earlier material had a predominance of cells with all chromosomes unpaired and those with bivalents were overlooked, while in the later material more cells had bivalents.

In the *capillaris-aspera* hybrid described by Nawaschin (1927) no bivalents occurred and none, some, or all of the univalents divided at the first division, the division having been initiated at diaphase. If all the chromosomes divided at first division a diad was formed, otherwise the second division appeared to proceed normally. It is very possible that some of the diads found in the *capillaris-tectorum* hybrids of this paper were formed in this way, but as was pointed out above a different mode of diad formation was observed. The initiation of univalent division at diaphase was not observed in *capillaris-tectorum* hybrids.

The meiotic behavior of the *capillaris-tectorum* hybrids is essentially similar to that described by Babcock and Clausen (1929) for three other interspecific hybrids in this genus. These hybrids involved only species with four chromosomes and each showed a variable number of bivalents with a maximum of four, the highest possible number.

## TRIPLOID HYBRIDS

(2 *n capillaris*, *n tectorum*)

Attention was drawn to the first of these triploid hybrids, which occurred among a number of normal  $F_1$  hybrids, by its higher percentage of good pollen and by its fertility, a considerable number of achenes per head having been formed while normal  $F_1$  hybrids had none or rarely one or two achenes per head. The plant was then caged and a number of selfed seed obtained from which progeny to be described later were derived.

When the next season's hybrids were grown some larger rosettes were set aside as being possible triploid hybrids and four of them proved to be so. Another plant which was selected at maturity, by its fertility proved on later examination to have been of the same chromosome constitution. Of the four of this group under observation from the rosette stage, only one produced any seed and from it a selfed progeny was obtained which will be discussed later.

The triploid hybrids resembled normal  $F_1$  (Hollingshead, MS) but showed the effect of the extra haploid *capillaris* chromosome complex in a slightly stronger resemblance to that species. The somatic cells showed clearly two haploid sets of *capillaris* and one of *tectorum* (fig. P, 1). As was shown by Nawaschin (1927) for similar hybrids the D-chromosome of *tectorum* lacks the satellite here as it does in normal  $F_1$ .

The first meiotic division showed almost always three bivalents and four univalents at diaphase (fig. P, 2) and metaphase (fig. P, 3). Presumably the three bivalents are formed from the diploid *capillaris* complex and the four univalents are the *tectorum* chromosomes. It is worth noting that whereas *capillaris* and *tectorum* chromosomes pair in the  $F_1$  hybrid the *tectorum* chromosomes here usually do not unite with the *capillaris* bivalents to form trivalents. Only rarely does one see six units at metaphase, one of which is probably a trivalent (fig. P, 4). Rarely, too, only two bivalents were formed and six univalents were to be seen. This lack of pairing within a diploid *capillaris* complex was noted above in the pure species.

At I-A the univalents segregate undivided or divide, the halves passing to either pole as shown in figure P, 5 and 6, their behavior having been determined apparently by their metaphase position, as

in normal  $F_1$  hybrids. Lagging chromosomes at II-A were common and tetrads with microcytes and micronuclei frequent. Though no extensive examination of second-anaphase figures was made, several cells showed a total of twenty chromosomes at this stage and it was

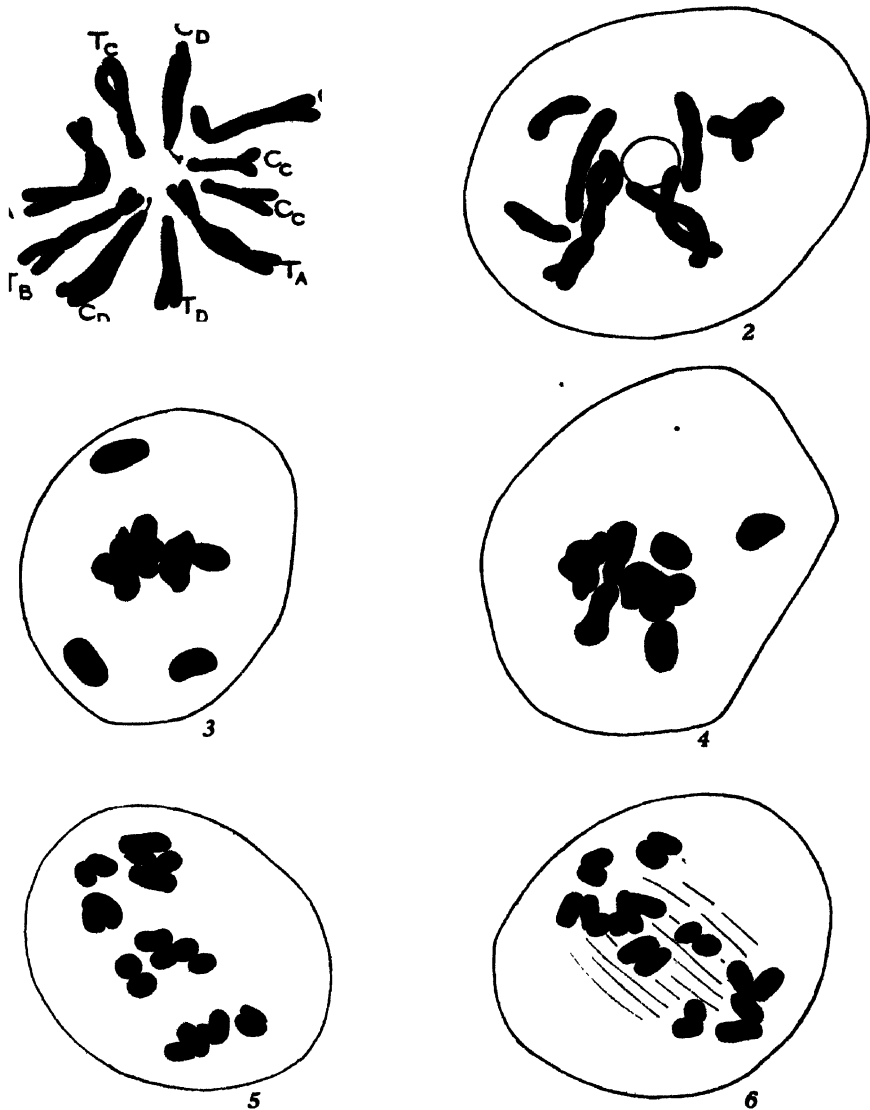


Fig. P. Triploid *C. capillaris-tectorum* hybrid, (1) somatic metaphase, (2) diaphase, (3) first meiotic metaphase showing three bivalents and four univalents, (4) first meiotic metaphase showing two bivalents, a trivalent, and three univalents, (5, 6) first meiotic anaphases showing both segregation and division of univalents.

definitely established that certain chromosomes segregated without division at second metaphase. In view of these observations and the behavior of  $F_1$  univalents it seems likely that univalent chromosomes

do not often, if ever, divide twice in this triploid hybrid. In spite of the fact that irregularities were more obvious in triploid hybrids than in most  $F_1$  hybrids examined, the percentage of good pollen was much higher, averaging from 17.2 to 28.8 per cent, and, as has been noted, three of them were somewhat fertile.

The meiotic behavior of the triploid hybrids,  $2n$  *capillaris*- $n$  *aspera*, described by Nawaschin (1927), showed three bivalents and four univalents but, unlike the *capillaris*-*tectorum* triploid hybrids, all the univalents divided at the first division, second divisions were regular, and almost always regular tetrads occurred. This would mean that the univalents divided at both divisions and that every gamete would have the somatic chromosome set. On this basis one would expect a high percentage of morphologically good pollen and practically complete fertility. Nevertheless very little good pollen was produced. No selfed progeny were secured by Nawaschin from his triploid hybrids.

## PROGENY OR TRIPLOID HYBRIDS

The meiosis of the triploid hybrids would lead one to expect that each male gamete (and presumably female) would contain a haploid set of *capillaris* chromosomes and one to four *tectorum* chromosomes. If the segregation of the *tectorum* univalents were a random one, fifteen-sixteenths of the gametes would possess at least one *tectorum* chromosome in addition to a haploid *capillaris* set. If all gametes were equally viable the zygotes derived from them would possess a diploid set of *capillaris* and none to eight *tectorum* chromosomes, i.e., a range of chromosomes number from six to fourteen. Table 6 gives the actual distribution of chromosome numbers in plants from the two

TABLE 6

THE ACTUAL DISTRIBUTION OF THE CHROMOSOMES NUMBERS OF THE PROGENIES OF TWO TRIPLOID *C. capillaris*-*tectorum* HYBRIDS COMPARED WITH THE THEORETICAL ONE

|                  | Chromosome number |    |    |    |    |    |    |    |    |
|------------------|-------------------|----|----|----|----|----|----|----|----|
|                  | 6                 | 7  | 8  | 9  | 10 | 11 | 12 | 13 | 14 |
| Progeny 1.....   | 10                | 5  | 2  | 1  | 1  | 1  | 1  | .. | .. |
| Progeny 2.....   | 49*               | 7  | 2  | 3  | 11 | 2  | 2  | 3  | 1  |
| Total.....       | 59                | 12 | 4  | 4  | 12 | 3  | 3  | 3  | 1  |
| Theoretical..... | 1                 | 8  | 28 | 56 | 70 | 56 | 28 | 8  | 1  |

\* Only nine of these plants were actually counted. The constitution of the others was inferred from the fact that they were morphologically typical *capillaris* plants.

selfed progenies compared with the theoretical one. The most outstanding feature is the difference between the actual and theoretical proportion of plants with six chromosomes only, i.e., with no *tectorum* chromosomes. They constitute more than half of one population by actual chromosome determination, and the same is doubtless true of the second population in which most plants which were morphologically like pure *capillaris* were not examined cytologically, whereas the theoretical expectation is only 1 in 256. The distribution of the chromosome numbers of the plants which contained some *tectorum* chromosomes differs from the theoretical, the seven-chromosome class being too high in both populations.

Were numbers alone taken into consideration one might postulate that gametes with *tectorum* chromosomes suffered in competition with those without, and those with one suffered least, thus giving rise to many zygotes with *capillaris* chromosomes only and a larger proportion with one *tectorum* chromosome than was expected. When the complexes of the plants were analyzed to determine exactly which chromosomes were present, however, and this was done for most plants, this explanation in its simplest form proved untenable. As expected, each plant did contain a diploid set of *capillaris*. According to chance combinations of the gametes derived from random segregation of univalents, many plants should have contained at least one pair of *tectorum* chromosomes. Of the ten-chromosome plants, for example, 97 per cent would have at least one *tectorum* chromosome represented twice and only 3 per cent would contain the four different *tectorum* chromosomes. Of the twelve plants with ten chromosomes all had the four different *tectorum* chromosomes. Indeed no *tectorum* chromosome was found twice in a plant unless all four were present once.

The results are explicable on the assumption that egg cells with most, or all, of the possible combinations of chromosomes may function but that only those pollen grains function which have either a haploid *capillaris* set or a haploid set of both species, each of which would occur once in sixteen times as a result of random segregation of univalents. The high percentage of poor pollen accords well with the hypothesis that considerable pollen is non-functional, although the proportion of obviously bad pollen is not high enough to account for the postulated elimination. Probably all well stained grains cannot function.

On this assumption, the constitutions of the female gametes which gave rise to particular plants may be determined. If the plant had

less than ten chromosomes, the gametic constitution of the egg from which it arose can be obtained by subtracting a haploid *capillaris* set from the whole chromosome complex. If the plant had ten chromosomes, the female gamete contained either a haploid *capillaris* set or haploid sets of both species. If it had more than ten, the gametic constitution of the egg from which it arose can be determined by subtracting the haploid sets of the two species from the whole complex. On this basis the distribution of functioning female gametes with one to three *tectorum* chromosomes in addition to the haploid *capillaris* set proves to be:

| Number of <i>tectorum</i> chromosomes . . . . . | 1  | 2 | 3 |
|---|----|---|---|
| Frequency. . . . .                              | 15 | 7 | 7 |

It is impossible to determine the frequency of functioning female gametes with four *tectorum* chromosomes since pollen grains of the same constitution presumably function also.

Since the two triploid hybrid progenies were apparently fundamentally similar in the distribution of chromosome numbers from which these calculations were made, they were grouped together for this purpose. In one detail, however, they were quite different, the second having a much higher proportion of ten-chromosome plants; and the difference seems to be too high to be ascribed to the small number of plants in each population. According to the hypothesis outlined above this might mean that one parent had produced a higher proportion of gametes with four *tectorum* chromosomes but there is no further evidence for such a deduction.

From those plants where it was possible to determine exactly which chromosomes were present, it was calculated that the distribution of various female gametic chromosome combinations had been as follows (using the chromosome designations as in fig. B):

| Gametic constitution   | Frequency  |
|--|------------|
| <i>n capillaris</i> +T <sub>A</sub> .....  | 6          |
| <i>n capillaris</i> +T <sub>B</sub> .....  | 3          |
| <i>n capillaris</i> +T <sub>D</sub> .....  | 1          |
| <i>n capillaris</i> +T <sub>A</sub> , T <sub>B</sub> .....                                   | 2          |
| <i>n capillaris</i> +T <sub>B</sub> , T <sub>D</sub> .....                                   | 1          |
| <i>n capillaris</i> +T <sub>B</sub> , T <sub>C</sub> .....                                   | 2          |
| <i>n capillaris</i> +T <sub>A</sub> , T <sub>B</sub> , T <sub>C</sub> .....                  | 1          |
| <i>n capillaris</i> +T <sub>A</sub> , T <sub>B</sub> , T <sub>C</sub> , T <sub>D</sub> ..... | At least 1 |

Besides the plants from which this distribution was calculated there occurred seven others each of which contained a chromosome which looked like  $T_A$  with a part of one arm missing (fig. Q, 1). These plants each had a diploid set of *capillaris*, a haploid set of *tectorum*, and this peculiar chromosome, with or without part of another haploid set of *tectorum* which never included  $T_A$ . The repeated occurrence of this chromosome, which may be a  $T_A$  from which a part has been lost by fragmentation and elimination at meiosis, might indicate that the A-chromosome of *tectorum* has a tendency to fragment at a certain point. The situation recalls that found by Nawaschin (1927) in the plants of the progeny of a triploid

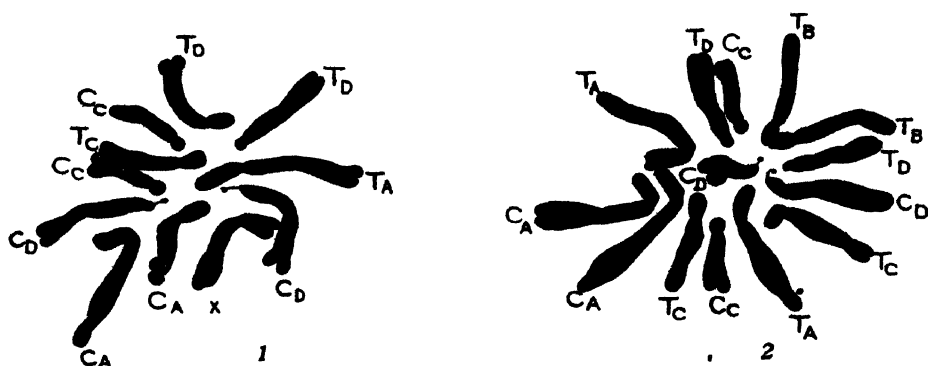


Fig. Q. Somatic metaphases of two derivatives of a triploid hybrid, (1) a plant with eleven chromosomes, one of which (x) is a type not found in the parental species (see text), (2) the amphidiploid derivative.

*capillaris-aspera* hybrid. Each had a typical A-chromosome of *capillaris* and one with a shorter arm than usual, which he interpreted as showing that hybridization had affected the shape of the chromosome. In later progenies of the same type, however, he was unable to find these modified chromosomes (unpublished data) and now inclines to the fragmentation hypothesis.

In two plants which had two D-chromosomes of *tectorum* one of these was satellited, the other was not. In all other cases each  $T_D$ -chromosome lacked the satellite, as in the  $F_1$  and the triploid hybrids. No explanation for the reappearance of the satellite was found.

A number of possible combinations of *tectorum* chromosomes are missing. The number of plants examined is too small to draw any conclusion from this fact but differential viability among the plants which did have different combinations favors the possibility that some chromosome combinations are quite inviable.

Each particular combination of *tectorum* chromosomes added to the *capillaris* complex had its peculiar effect on the morphology of



the plant. All plants with a diploid *capillaris* complex and part of a haploid *tectorum* set were less viable than plants with only *capillaris* chromosomes, and the plants with two *tectorum* chromosomes were usually less viable than those with one or three. Plate 2, *a*, shows a typical *capillaris* plant at the rosette stage and three others, each of which had a different *tectorum* chromosome added to the *capillaris* complex.  $T_B$  was most injurious to viability. Plate 2, *b*, shows four plants with more than one *tectorum* chromosome added to the *capillaris* complex. The combination of  $T_A T_B$  with *capillaris* was the least viable one obtained. Those which flowered under the writer's observation were sterile.

Plants having ten chromosomes, with one exception, resembled the triploid hybrid from which they arose. The one exception was the single plant in the first population which had ten chromosomes. It was rather weak and sterile, and differed in habit and leaf shape from its parent. The chromosome complex showed a diploid set of *capillaris*, A-, B-, and C-chromosomes of *tectorum* and a satellited chromosome which could not be distinguished from the *capillaris* satellited chromosomes. Probably non-disjunction or non-conjunction in the *capillaris* set had given rise to an egg with two *capillaris* satellited chromosomes instead of the usual one.

Plants with more than ten chromosomes formed vigorous rosettes. Only the first population was under the writer's observation during the whole life of the plants and the one plant with twelve chromosomes never flowered. The other with eleven was sterile. Some of those in the second population were in flower when the writer had to discontinue the investigation, others were still at the rosette stage. They showed morphological differences but were more uniformly viable than those with fewer than ten chromosomes.

The possibility of hybridization having played a part in the evolution of chromosome numbers within this genus has been suggested by Nawaschin (1925*b*) and Hollingshead and Babcock (1930). The plants just described, however, constitute evidence against the hypothesis which would explain the origin of a species with a higher chromosome number by the addition of a pair of chromosomes from another species to the complex of an existing one following hybridization. As has been pointed out, the addition of a single chromosome of *tectorum* to the *capillaris* complex lessened viability and caused sterility. It does not necessarily follow, of course, that the same would hold good for derivatives of other interspecific hybrids within the genus.

## THE AMPHIDIPOID DERIVATIVE

The nature of the progeny of the first triploid hybrid favored the possibility of obtaining a plant with a diploid chromosome complex of each species, and it was largely in the hope of obtaining such a plant that the second population was grown and examined. When such a fourteen-chromosome plant was obtained, disappointingly enough, although containing the expected chromosome constitution, it was somewhat spindly and weak compared with the stouter, though later, *capillaris* and triploid hybrid plants of the same population (pl. 3). It produced no typical rosette but sent up a weak flower stalk very early. Many heads were abnormal with greenish yellow split ligulae and no pollen, a phenomenon occasionally met with in  $F_1$  hybrids. Some heads however, were normal in appearance and in other characters it resembled a normal *capillaris-tectorum*  $F_1$  hybrid. Part of the weakness was doubtless due to its being grown in the winter, but this characteristic was too marked to be attributed to environment alone.

TABLE 7  
FIRST METAPHASE COUNTS FROM THREE BUDS OF THE AMPHIDIPOID  
*C. capillaris-tectorum* HYBRID

|            | 7" | 6"+2' | 5"+4' | 4"+6' |
|------------|----|-------|-------|-------|
|            | 6  | 2     | 2     | 1     |
|            | 6  | 7     | 2     | ....  |
|            | 10 | 9     | 4     | ....  |
| Total..... | 22 | 18    | 8     | 1     |

The somatic chromosome complex is shown in figure Q, 2. Each *tectorum* D-chromosome lacks its satellite. Meiotic divisions showed the expected seven pairs in less than half of the cells. Table 7 shows the frequency of the combinations of bivalents and univalents observed in three slides. Since the higher the number of units the more difficult it is to distinguish the exact number of bivalents and univalents, it is probable that the irregularities were more common than the table indicates. Figures R and S show first diaphase and metaphase stages illustrating the various combinations observed and one anaphase showing a regular distribution of bivalent partners. Tetrads showed occasional microcytes and micronuclei. One normal

head gave 32.4 per cent apparently good pollen. The high percentage of irregularity and the sterility is disappointing, for hopes had been entertained that this would constitute an experimental verification of an hypothesis which would account for some of the various chromosome numbers of the species within the genus by hybridization fol-

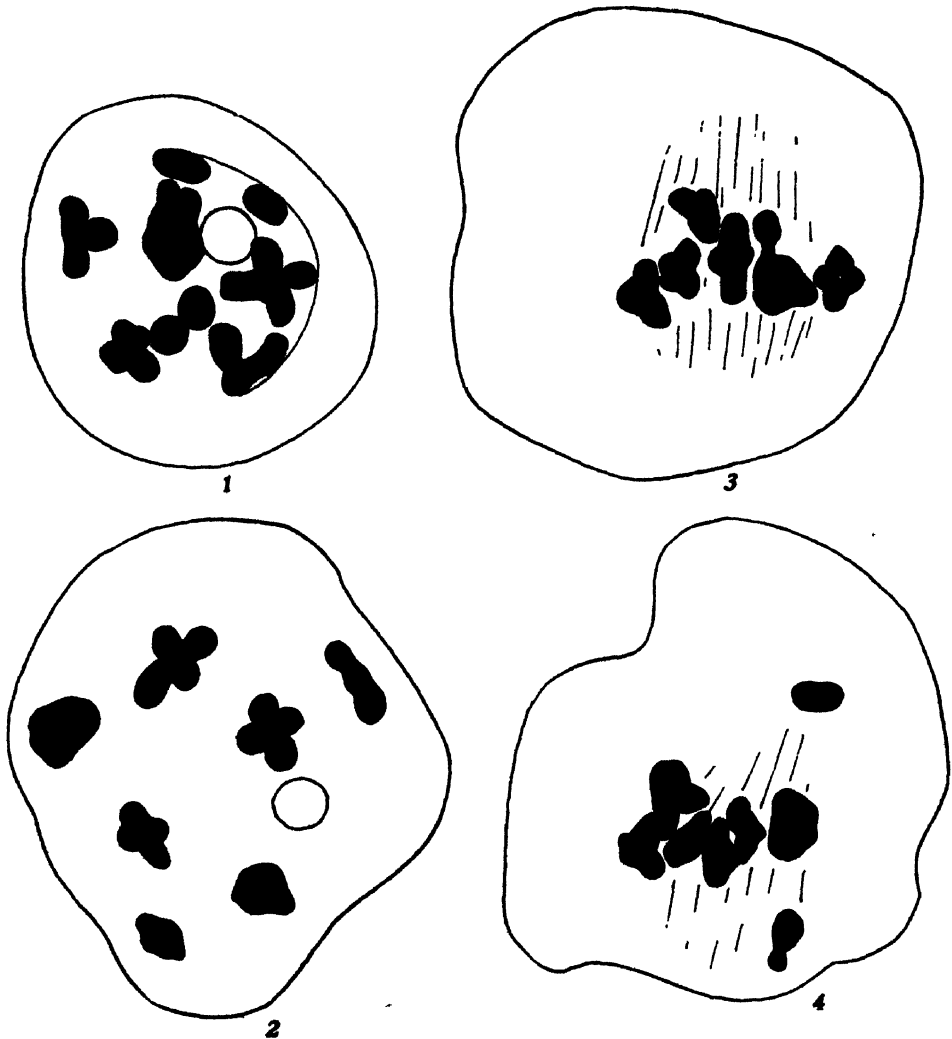


Fig. R. First meiotic divisions of the amphidiploid derivative, (1) diaphase with six bivalents and two univalents, (2) with seven bivalents, (3) metaphase with seven bivalents, (4) with six bivalents and two univalents.

lowed by chromosome doubling (Hollingshead and Babcock, 1930). That the irregularities can be attributed wholly, or even largely, to environmental conditions seems unlikely since some *capillaris* plants growing under similar conditions had regular meiotic divisions. It seems very probable, however, that the meiotic irregularities are

expressions of the same phenomenon as that met with in the X-strain of *capillaris* which is the strain incorporated in this amphidiploid hybrid. Under the circumstances it would be premature to conclude that similar hybrids would always be weak, meiotically irregular, and sterile, or to abandon the hypothesis that such hybrids may have figured in the evolution of the chromosome numbers in the genus.

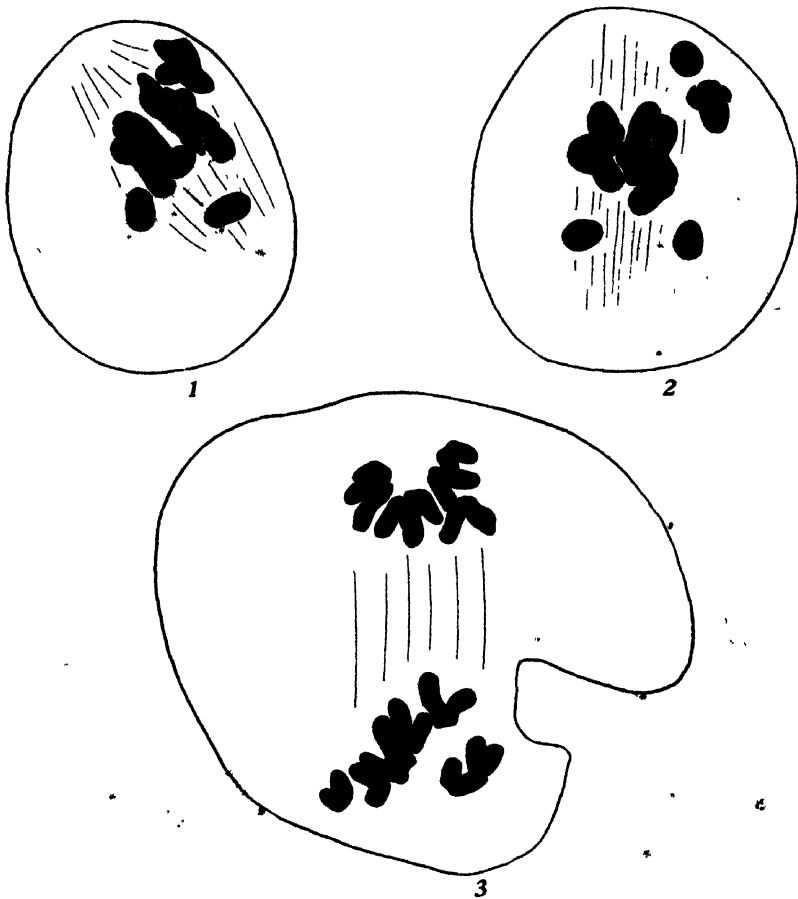


Fig. 8. (1, 2) First meiotic metaphases with five and four bivalents respectively, (3) regular first anaphase.

Nevertheless, in this connection it is worth noting that an amphidiploid *C. capillaris*-*C. dioscoridis* hybrid obtained by M. Nawaschin (unpublished data) was smaller than  $F_1$  hybrids of these two species and was sterile.

Amphidiploids (also called didiploids, allopolyploids, and tetraploid hybrids) which involve two species of the same genus have been described in *Nicotiana* (Clausen and Goodspeed, 1925, Eghis, 1927, and Rhybin, 1927), *Rosa* (Blackburn and Harrison, 1924),

*Prunus* (Crane and Darlington, 1927),<sup>1</sup> *Saxifraga* (Marsden-Jones and Turrill, 1928), *Solanum* (Jorgenson, 1928), *Digitalis* (Buxton and Newton, 1928), and *Primula* (Newton and Pellew, 1929). Similar combinations which involve the chromosomes of species of different genera have been described in *Aegilops-Triticum* hybrids by Tschermak and Bleier (1926), and in *Raphanus-Brassica* hybrids by Karpechenko (1927). As pointed out by Newton and Pellew, there are two known methods by which these have arisen from normal  $F_1$  hybrids—the doubling of chromosomes in the soma of the  $F_1$  hybrid and the production of unreduced gametes which give rise to amphidiploids directly or to triploid hybrids which subsequently give rise to amphidiploids. The derivation of the *Crepis* amphidiploid is of interest in that it, like the *Nicotiana* of Eghis and Rhybin, arose from a triploid hybrid which was the result of a union of an unreduced gamete from a pure species with a normal gamete from another. Thus the formation of the amphidiploid can be traced to the occurrence of non-reduction in *Crepis capillaris*.

Amphidiploids generally have been at least as vigorous as the  $F_1$  hybrids from which they arose. The spindly nature of the *capillaris-tectorum* amphidiploid is particularly difficult to understand in view of the fact that  $F_1$  hybrids and triploid hybrids involving these species are vigorous and healthy, except when an  $F_1$  hybrid receives a lethal factor from its *tectorum* parent (Hollingshead, MS). While the amphidiploids which have been examined meiotically have shown small percentages of irregularities, in no other case has as high a percentage as that which characterized the *Crepis* amphidiploid been reported. One is more inclined to attribute the high percentage of irregularity of this amphidiploid to the *capillaris* portion of the amphidiploid chromosome complex than to any effect of the combination of the two specific chromosome garnitures.

<sup>1</sup> Actually a hybrid between tetraploid and diploid species, which arose from an unreduced gamete of the latter, giving rise to a plant with four haploid complexes.

## SUMMARY

1. Examination of somatic chromosomes of the  $F_1$  hybrid of *C. capillaris* ( $n=3$ ) and *C. tectorum* ( $n=4$ ) corroborated Nawaschin's observation that the satellite on one chromosome of *tectorum* was not to be found in the hybrid. Measurements of the various chromosomes indicated that in the reciprocal hybrids examined another *tectorum* chromosome was shorter than in the parental species.

2. Meiotic divisions in *tectorum* proceeded normally but plants of the X-strain of *capillaris* frequently exhibited irregularities in the form of non-conjunction and irregular distribution of chromosomes. In extreme cases scarcely any good pollen was found.

3. Variation in number of bivalents at first meiotic metaphase characterized all  $F_1$  hybrids. In hybrids involving one strain of *tectorum* three bivalents and one univalent was the most frequent combination and seven univalents were very rare. In those involving a second strain the frequencies of the various possible combinations of bivalents and univalents were approximately equal with three bivalents and one univalent the least frequent combination. The suggestion is offered that some such difference in hybrids involving different strains of a particular species may explain why Collins and Mann at first found no pairing in *C. capillaris*-*C. setosa*  $F_1$  hybrids, and later Mann Lesley found as many as three bivalents in similar hybrids involving different strains.

4. The univalents in the *capillaris*-*tectorum* hybrids divided either at the first or second division, but not at both. The hybrids were almost completely sterile.

5. Several triploid hybrids which contained a diploid set of *capillaris* and a haploid set of *tectorum* were found among ordinary  $F_1$  hybrids and selfed progenies were obtained from two of them. The *capillaris* chromosomes united to form three bivalents at meiosis leaving the *tectorum* chromosomes unpaired. The chromosome constitutions of the progeny indicated that only male gametes which had none, or all, the *tectorum* chromosomes functioned but that most, or all, of the female gametes could function. Thus plants were secured with only *capillaris* chromosomes and with various combinations of *tectorum* chromosomes added to a *capillaris* complex. With the

exception of derivatives of the parental constitution (triploid hybrids) those with *tectorum* chromosomes were less viable than those without. This constitutes evidence against a theory which would account for an increase in chromosome number during the evolution of the genus by the addition of a pair of chromosomes from another species following hybridization.

6. One amphidiploid derivative with diploid chromosome complexes of each species was secured. It was less vigorous than triploid hybrids of the same population but matured earlier. Meiotic irregularities were very frequent and a large proportion of the pollen was bad, and it proved to be completely sterile. No explanation for its lack of vigor was found, but it may be that the meiotic irregularities were introduced by *capillaris* which was of the frequently meiotically irregular X-strain. Under the circumstances it would be premature to infer that the lack of vigor and the meiotic irregularity of this plant was evidence against the hypothesis which would account for some of the chromosome numbers within the genus by the occurrence of amphidiploidy.

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PLATE 1

Typical plants of *C. tectorum*, F<sub>1</sub> *C. capillaris-tectorum*, and *C. capillaris* at maturity.

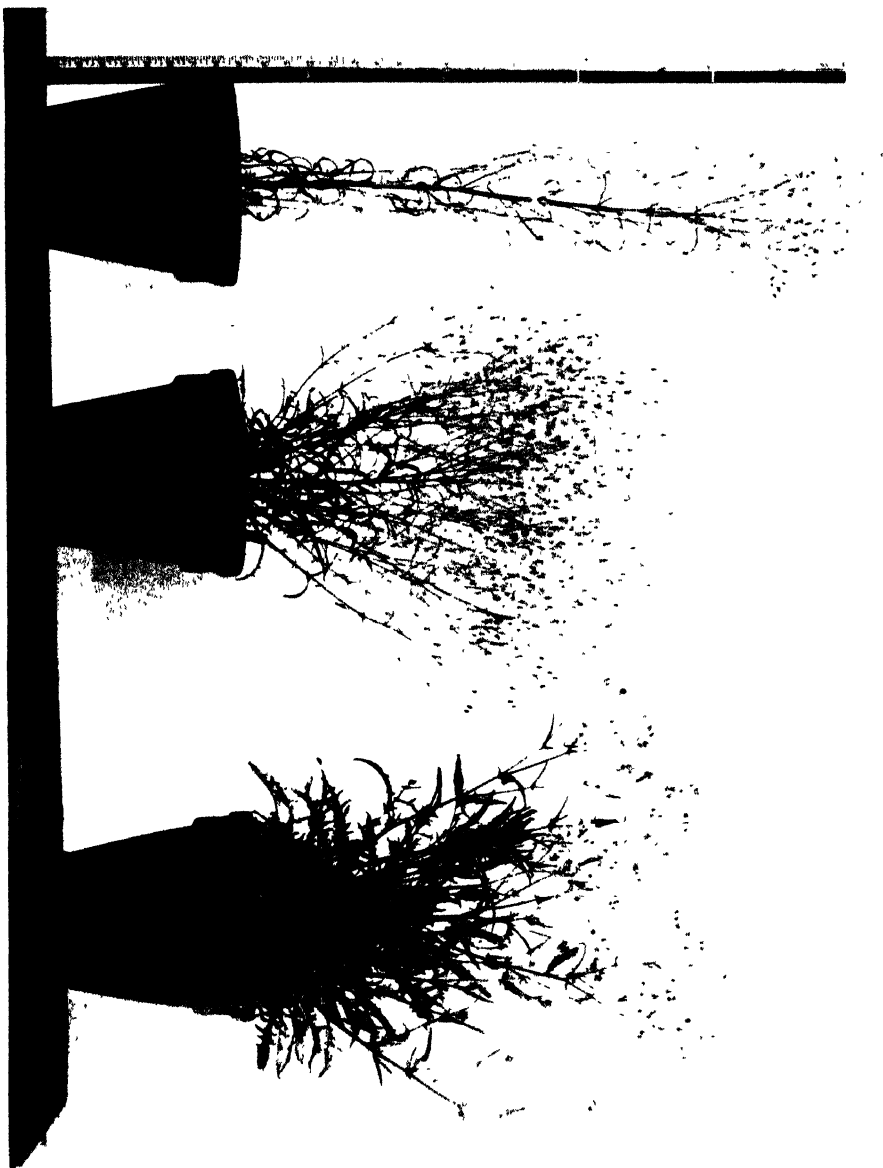


PLATE 2

Plants of the progeny of a triploid hybrid showing the effect of various chromosomes of *C. tectorum* added to the *capillaris* complex.

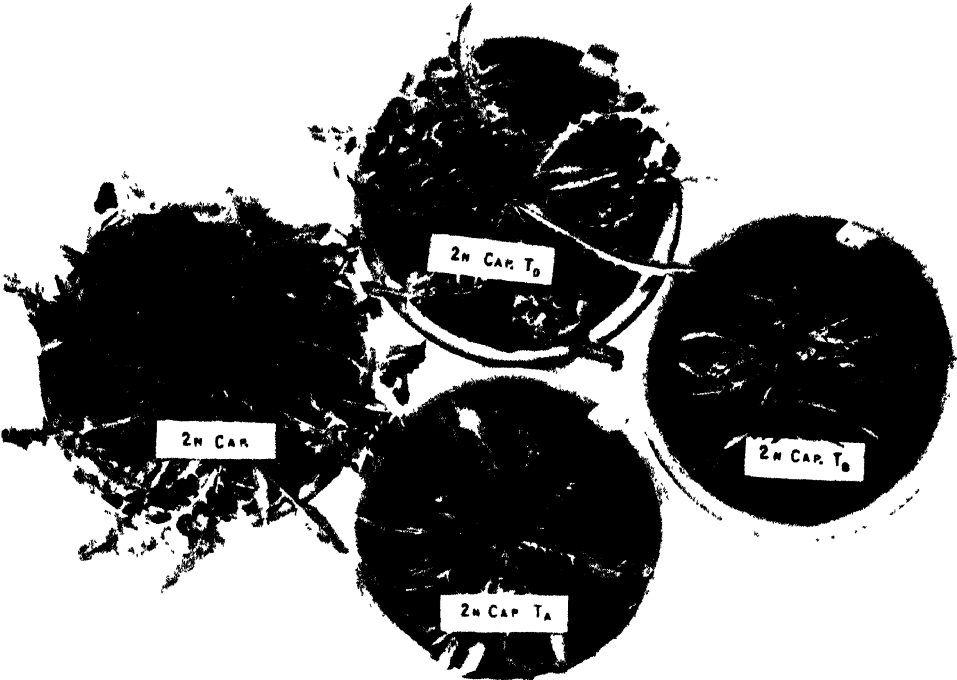


PLATE 3

Triploid hybrid progeny. From left to right, *C. capillaris*, amphidiploid, and triploid hybrids.



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**UNBALANCED SOMATIC CHROMOSOMAL  
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# UNBALANCED SOMATIC CHROMOSOMAL VARIATION IN CREPIS

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M. NAVASHIN

## INTRODUCTION

It is a well-known phenomenon that the chromosomal constitution may vary during the somatic cycle of development. Numerous instances have been described for various representatives of the two organic kingdoms. The majority of such phenomena may be divided into two principal groups, viz., *chromosomal chimeras* and *chromosomal variegation*. In chromosomal chimeras, entire shoots, branches, groups of similar tissues, different tissues, or finally, groups of cells within the same tissue, differ in their chromosomal composition. Various kinds of chimeras may be of occasional occurrence, whereas in certain cases a chimera condition constitutes a constant typical feature of the organism. Thus, the tetraploid shoots known to occur in *Datura* (Blakeslee and Belling, 1924) and the so-called mosaics in *Drosophila* (Morgan, Bridges and Sturtevant, 1925) represent chimera conditions of occasional occurrence. Constant differences in the chromosomal constitution of different tissues of certain insects (Frolowa, 1928) or in the root tips of hemp (Breslawetz, 1926) may be referred to as typical features of certain organisms.

Extreme chimera conditions, in which separate little groups of cells of different chromosomal constitution are occasionally found in normal tissues, represent transitional stages to chromosomal variegation. The distinction between the two kinds of variation depends obviously upon the degree of mutability. If variation occurs infrequently, the resulting chromosomal constitution becomes fixed in long lineages of cells and a chimera arises. If, on the contrary, the frequency of variation is very high, no definite lineage of cells can possibly arise, and variegation is the result.

In *Crepis* investigations, many cases of vegetative chromosomal variation have been observed, the great majority of them being located in the root tips. They were mostly of the balanced type, i.e., they represented various grades of polyploidy of definite sectors of cells or

of individual cells. Tetraploid cells were found, for instance, in the root tips of *C. dioscoridis* (M. Navashin, 1926) and of a hybrid derivative of *C. biennis*  $\times$  *C. setosa* (Hollingshead, 1928a). Giant polyploid cells containing as many as  $128n$  chromosomes were found in *C. tectorum* (M. Navashin, *loc. cit.*). Many instances of various grades of polyploidy of chimera type were found by the present writer and by Miss Gerassimova in several *Crepis* species and in interspecific hybrids. Finally, it was found in haploid individuals of *C. capillaris* (Hollingshead, 1928b; Babcock and Navashin, 1930) that a very considerable proportion of cells become diploid, the latter circumstance even leading to the formation of diploid shoots (Hollingshead, *loc. cit.*).

As was stated above, the majority of observations concern the root tips. It was found many times by the present writer that different roots taken from the same plant differed in their chromosomal constitution. Thus in many instances entire tetraploid roots were found to occur, together with normal ones, in diploid individuals of *C. tectorum* and *C. capillaris*. Furthermore, in plants possessing small extra fragments or other chromosomal abnormalities, certain roots were found to be entirely normal as regards their chromosomes. Finally, cases were found when a single plant yielded roots of three different chromosomal types; and hybrid derivatives possessing one foreign chromosome produced some roots without that extra chromosome. All these and similar observations, however, were mostly explained as experimental errors, i.e., as due to occasional confusion of roots belonging to different individuals, a circumstance eventually taking place. All these cases, however, could by no means be explained as mere results of errors. On the contrary, by taking special precautions it was found that the majority, if indeed, not all of them should be attributed to actual variation in somatic cycle.

In the cultures of the past year (1929) one plant was found which was undoubtedly of unbalanced chimera type. Not only its roots but the upper parts of the shoots as well differed in their chromosomal constitution. The description of this interesting chimera, the discussion of its mode of origin and of the bearing of chromosomal variation on some problems of general importance constitute the principal subject of this paper.

It gives the writer special pleasure to acknowledge the excellent help given during the course of the investigation by Miss H. N. Gerassimova.

## AN UNBALANCED CHROMOSOMAL CHIMERA IN *C. tectorum* L.

Numerous progenies of triploids in *C. tectorum* have been grown and subjected to investigation by the writer since 1926. Among several cultures grown in 1929 in Moscow in the experimental garden of the Timiriazev Federal Institute of Scientific Research, one, namely culture 29.1013, contained the plant which is described below. This particular plant 29.1013-40 was investigated cytologically, and root tips were taken from it in the young rosette stage. Altogether twenty-six roots were investigated. Nineteen of them proved to be triplo-B simple trisomic, the remaining seven showing wholly normal diploid

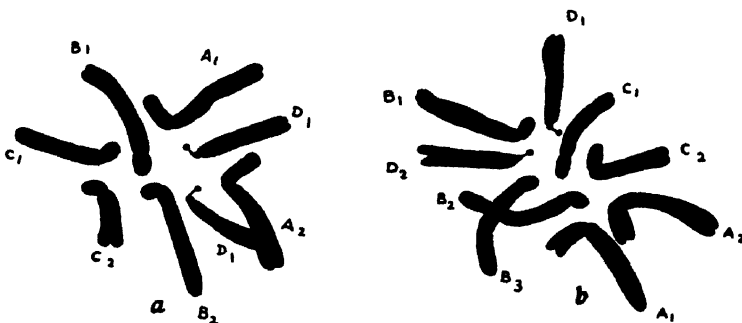


Fig. 1. Somatic metaphases: *a*, of the normal (diploid) component; *b*, of the trisomic component (note the extra B-chromosome). Root tips.  $\times 2400$ .

chromosomal complement (fig. 1). This first result of the investigation strongly suggested the conclusion that the plant in question was a sectorial chimera with one part trisomic and the other normal diploid.

Further observations have shown the correctness of this conclusion. After the shoots had developed it appeared that they were of two clearly distinct types. Two of them were undoubtedly triplo-B simple trisomic ("diamond"), the third being of typical normal appearance. The trisomic shoots were (as is common for trisomies) about half as tall as the normal component and possessed the typical "diamond-shaped" leaves which are the best morphological characteristics of the triplo-B type. The flowering heads, buds, and fruiting heads were much smaller in the dwarf component as compared with the tall one. This difference in head size is again a constant distinction between the triplo-B trisomies and normal plants. The cytological investiga-

tion of the young meristem of the flower buds and of the root tips, taken separately from the two components, proved beyond any question that the plant really was a trisomic-normal, sectorial chimera. Thus nine chromosomes were found in the buds and root tips of the dwarf component whereas those of the tall component had only the four pairs, typical of the somatic garniture in *C. tectorum*. (See pls. 4 and 5 and fig. 1.)

As noted above, the two components of the chimera did not differ morphologically in any way from the respective entire plants of corresponding chromosomal constitution. With regard to their physiological behavior, the trisomic portion did not show any marked peculiarities, but the "normal" diploid component was entirely different from the thousands of diploid *C. tectorum* plants that had been previously seen. In contrast to them, it was of *very low fertility*, a phenomenon never met with in normal individuals of *C. tectorum*. The cause of this low fertility of the "normal" component could not be detected. No irregularities in meiosis in P.M.C. were found.

### MODE OF ORIGIN OF THE CHIMERA

The chimera described above occurred in the progeny of a triplo-A simple trisomic plant derived from a triploid parent. The appearance of an extra B-chromosome should be attributed, therefore, to elimination of the extra A-chromosome followed by duplication of the B-chromosome, most likely through equational non-disjunction. Cases of the latter sort were observed many times by the writer when certain chromosomes in hybrids were duplicated, obviously through equational non-disjunction. As for the origin of the chimeral condition, two principal ways are feasible; first, through diembryony, and second, through somatic elimination of the extra chromosome sometime during early development; at any rate prior to the formation of flowering shoots. The first manner of origin is highly improbable as might be easily shown. Thus, in order to account for the occurrence of two cytologically different embryos in the same achene, one may suggest the three following possibilities: the formation of an adventitious embryo from the vegetative tissue, or of two functional embryo sacs in the same ovule, or finally of two functional ovules in the same ovary. Occurrence of adventitious embryo is a phenomenon never met with in *Crepis* and, furthermore, it could by no means account

either for the formation of a normal diploid embryo or for that of a triplo-B trisomic. It could give rise only to an individual identical with the original plant, i.e., a triplo-A trisomic. Two embryo sacs in the same ovule occasionally occur in *Crepis*; likewise instances have been found where a double ovule is formed. In such cases, however, the second embryo sac is situated at the chalazal end of the ovule and could hardly be fertilized, for the simple reason that the pollen tube could never reach it. Also, the double ovules are most probably incapable of development; never were embryos found to be contained in them.

Thus it seems that the most probable way to explain the origin of the chimera is to admit the somatic elimination of the extra chromosome which could give rise to the normal diploid shoot.<sup>1</sup> Unbalanced somatic chromosomal variation is known to occur in *Datura* (Blakeslee and Belling, *loc. cit.*) and is strongly suspected in many other cases.

With regard to the mechanism of somatic chromosome elimination, some observations of the present writer on phenomena found in root tips in *C. tectorum* may throw light on the problem. The results of these observations are given below.

### CHROMOSOMAL VARIEGATION in *C. tectorum*

Among several hundred seedlings grown from seed obtained from a normal *C. tectorum* plant, one was found that showed a striking peculiarity, namely, the meristematic cells of its root tip differed in various ways one from another in their chromosomal constitution.

Furthermore, the most outstanding features of this individual was that its chromosomes not only differed in number in different cells of the root tip but that some of them were atypically built. These atypical chromosomes, instead of being rod-shaped, were the shape of *rings* or *disks*. In early prophase they appeared like broad rings formed by a thread of the same diameter as the remaining normal chromosomes of the same nucleus. In later stages the rings became progressively contracted while thickening of the thread and narrow-

<sup>1</sup> One could suppose, on the contrary, addition of the third B-chromosome leading to the formation of trisomic shoots by a normal diploid plant. The results of cytological investigation have shown, however, that the majority of the roots were trisomic (see above). Moreover, two shoots were trisomic and only one, diploid. All this makes it most probable that the plant in question was originally a triplo-B trisomic and that in the course of development it produced one diploid shoot through elimination of the extra chromosome.



ing of the central clear space took place. At the metaphase the space within the rings usually disappeared entirely so that the rings assumed the form of more or less regular disks. In no stages of development did these structures differ in their staining reactions from the ordinary chromosomes. In one case the two daughter strands composing a normal chromosome failed to separate in the anaphase. In the few telophases observed, the atypical chromosomes resulted in two identical

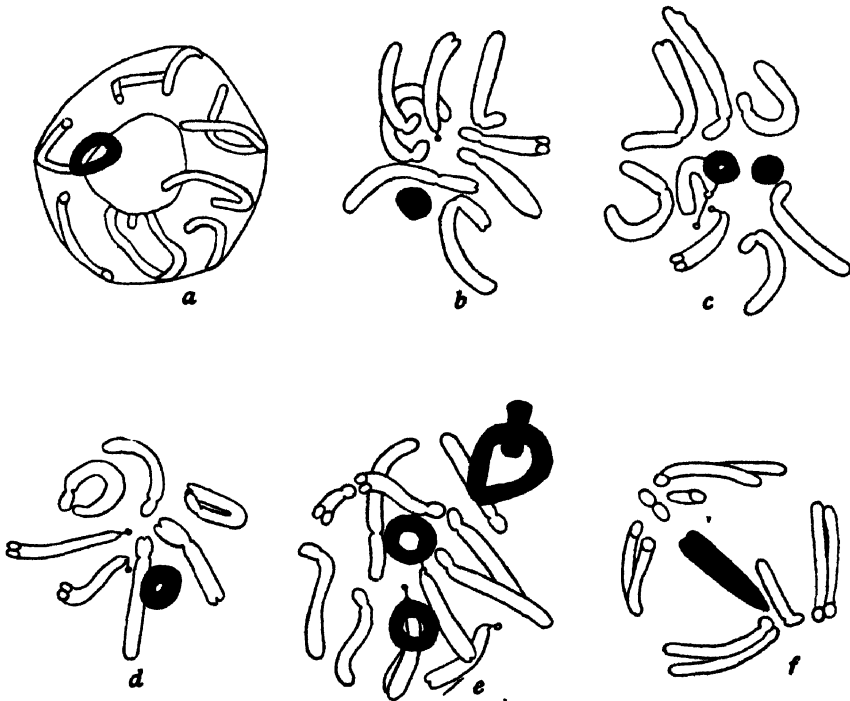


Fig. 2. Chromosomal variegation in *C. tectorum*.  $\times 2400$ .

a, prophase showing one "ring"; b, metaphase showing eight chromosomes plus one "disk," note the absence of the second satellited chromosome, which has been either transformed into the "disk" or replaced by a non-homologous chromosome; c, metaphase showing normal diploid complement plus one "ring" and one "disk"; d, metaphase showing seven chromosomes plus one "ring"; e, incomplete tetraploid metaphase showing four "rings," two of them united in a fashion of chain links; f, late anaphase showing one lagging chromosome which failed to divide into daughter halves.

bodies situated like the ordinary telophasic chromosomes at the two opposite poles of the cell. Thus there was hardly any doubt that one was dealing with real chromosomes modified in their organization in such a way that the ends of the chromosomes, instead of being free, became united thus forming a closed ring.

These peculiar chromatin structures varied in number in different cells. The number of the remaining normal chromosomes varied as

well. There were found cells which contained normal diploid chromosome sets plus one or two ring-shaped chromosomes extra; those containing nine normal chromosomes plus one "ring" extra; those with seven normal chromosomes and one "ring" extra. Finally, polyploid (probably tetraploid) cells were found which contained a varying number of "rings" along with the normal chromosomes; sometimes there were as many as five "rings." In addition to all these cases of variable chromosomal composition, cells with entirely normal diploid complement were not infrequent. (See fig. 2.)

The distribution of the cells of different chromosomal constitution appeared to be more or less a random one. It was evident that the process of chromosomal variation in this plant took place frequently and probably often in opposite directions in subsequent generations resulting from the same cell. Consequently no long lineage of cells of identical chromosomal constitution could possibly be established, and a typical variegation resulted.

## DISCUSSION

On the basis of the above data, it may be considered as proved that unbalanced somatic chromosomal variation actually takes place in *Crepis*. The case described of chromosomal variegation in *C. tectorum* shows that some disturbances in the chromosomal mechanism may lead both to addition and subtraction of individual chromosomes even in cells closely related by descent. Although only one actual case of failure of daughter chromosomes to disjoin was observed (cf. fig. 2f), the occurrence of cells of different chromosomal constitution in the immediate progeny of the same cell indicates that such or some similar process must be not infrequent. Otherwise one is compelled to admit the possibility of the formation of chromosomes *de novo* or of the disappearance of same, a statement which is against all cytological knowledge. On the contrary, the most probable assumption is that some chromosomal disorder, like that described above, makes it impossible for the chromosomes to function normally, and leads from time to time to addition (duplication) or to subtraction of certain chromosomes. These conditions may arise only for a short period of time, perhaps even only in one cell and may lead to the formation of long lineages of cells of aberrant chromosomal constitution. A chimeral individual may arise in such a case. If, on the contrary, the dis-

turbance persists in the tissue, no definite chromosomal constitution can be established and variegation takes place.

The phenomenon of somatic chromosomal variation throws some light upon certain problems of general interest. Thus it is very probable that occasional addition or subtraction of individual chromosomes may be responsible for "bud variation" of various kinds as has been suggested by various writers (cf. review by Chittenden, 1927). Balanced chromosomal variation may represent a legitimate condition of specialization of certain tissues. Somatic variation of high frequency like that described above for *C. tectorum* may result in chromosomal variegation. And, if genes of visible characters are involved in it, genetic variegation would be the result. In certain cases true somatic segregation may take place, namely, if one allelomorphous chromosome be replaced by another through the addition of one chromosome followed by the subtraction of another. Thus homozygous branches may eventually be produced by a heterozygous individual without any "reverse gene mutation" at all.

One feature of the described chromosomal chimera deserves special emphasis. As was pointed out above, the "normal" component possessed a greatly reduced fertility in spite of its normal chromosomal composition and completely healthy condition. It should be concluded, therefore, that the trisomic component exerted some influence on the "normal" component that led to the reduction of its reproductive power. This clearly indicates that not only the similarity or dissimilarity of the genes contained in the allelomorphous chromosomes but some other internal conditions as well may control fertility. Investigation is in progress.

## SUMMARY

1. Among many plants of a triploid progeny of *Crepis tectorum* L. one sectorial chromosomal chimera was found. It consisted of three shoots, two of which were triplo-B simple trisomic ("diamond"), the third being normal diploid. The trisomic component did not differ in any way from the complete trisomic plants of the corresponding type. The "normal" component was morphologically identical with the ordinary diploid plants of the same species but differed from them in that its fertility was greatly reduced.

2. A case of "chromosomal variegation" in *C. tectorum* is described. This phenomenon consists in a highly frequent variation in chromo-

somal constitution leading to formation of scattered cells differing in various ways in their chromosome contents. The process of chromosomal variation goes on here in either direction, i.e., either addition or subtraction of definite chromosomes takes place in subsequent cell generations.

3. The origin of the described sectorial chimera is explained by loss of the extra B-chromosome early in development. The variation in chromosomal composition took place only once, thus establishing a lineage of cells of new chromosomal type. Frequent variation making it impossible for the cell progenies to establish certain definite stable chromosomal constitution leads to variegation.

4. It is suggested that somatic chromosomal variation may lead under some circumstances to "bud variation" or even to true somatic segregation if one allelomorphic chromosome of a heterozygous individual is replaced by another through a process similar to that described for the "variegated" individual of *C. tectorum*. Genetic variegation may possibly also depend upon chromosomal variegation.

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#### PLATE 4

General view of the chimera 29.1013-40 of *Crepis tectorum* L. Two trisomic shoots (left) and one normal shoot (right). Note the difference in the leaf shape, in the habit of growth, and in the general development of the two components. The plant was taken from the ground just before the photograph was made. Natural size *circa*.



### PLATE 5

Some details of the same chimera plant. Below, the lower part of the chimera showing the robust stem of the normal component and the weak slender stems of the trisomic component. The mode of connection between the two components clearly indicates that the formation of the diploid (normal) shoot must have taken place very early in the development.

Above, buds, flowering and fruiting capitula of the trisomic component (left) as compared with those of the normal component (right). Natural size *circa*.





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**BY**

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# A CYTOLOGICAL STUDY OF HAPLOID CREPIS CAPILLARIS PLANTS

BY

LILLIAN HOLLINGSHEAD

In a preliminary note (Hollingshead, 1928) the occurrence of two haploid plants of *Crepis capillaris* ( $n=3$ ) was reported, in populations of *C. capillaris*—*C. tectorum*  $F_1$  hybrids. In the course of the same experiment three other similar haploids were found. Shortly after the writer found the first two haploids, Dr. M. Navashin, working in the same laboratory, found a similar one in the progeny of a *C. capillaris*—*C. neglecta*  $F_1$  hybrid which had been open-pollinated (unpublished data). Unfortunately it died at the rosette stage. The same summer another *C. capillaris* haploid was found by Mr. C. W. Haney of the laboratory staff in a population resulting from crossing *C. capillaris* and *C. setosa*, and its haploid nature was established by the writer. Four of the writer's five haploids reached maturity and these with the one obtained by Mr. Haney furnished material for meiotic as well as mitotic studies. Observations were also made on the morphology and fertility of these haploids. The results of the studies differ in certain aspects from observations on haploid plants of other genera hitherto reported by various writers.

## ACKNOWLEDGMENTS

Most of the investigation was carried out while the writer was research assistant to Professor E. B. Babcock, and her indebtedness to him for providing the opportunity of pursuing the study and for his continuous interest and helpful advice is gratefully acknowledged. Thanks are also due Mr. C. W. Haney for permission to include in the study the haploid found by him.

## OCCURRENCE AND APPEARANCE OF THE HAPLOID PLANTS

The haploids developed from apparently normal achenes produced on heads of *C. capillaris* which had been emasculated and pollinated with pollen of another *Crepis* species, as described by Hollingshead (1930b). Five of them appeared in populations of *capillaris-tectorum* hybrids totaling over three thousand plants in an experiment extending over two years, and the sixth arose from one of two achenes produced on a *C. capillaris* head pollinated with pollen of *C. setosa*. Both *tectorum* and *setosa* have four pairs of chromosomes.

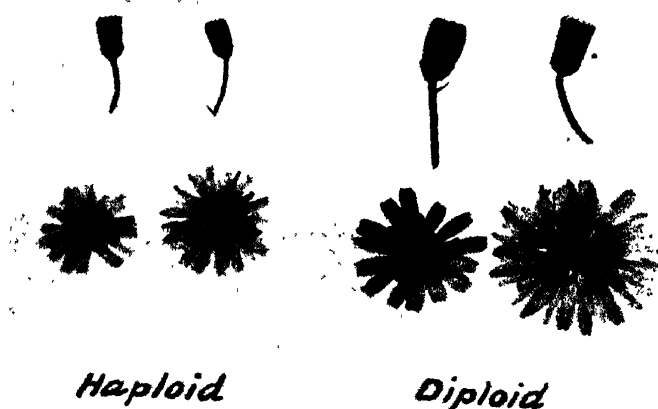


Fig. 1. Buds and heads from haploid and diploid *Crepis capillaris* plants. Natural size.

Attention was drawn to the haploids first determined by their appearance and by the fact that one of them was a viable plant in a population of hybrids which were dying at the cotyledon stage (Hollingshead 1930b). The rosettes, even at an early stage, were smaller, more compact, and flatter than normal ones. The leaves were smaller and the margins were less deeply indented, as shown in plates 6a and b. Chromosome counts from root tips established their haploid nature, and the other haploids were then picked out by their similar appearance, or in one case by flower size and sterility.

In habit and leaf shape, the mature plants resembled reduced diploids with fewer leaves. The branches tended to be slender and

usually the first branch emerged from the side of the crown rather than from the apex, as normally occurs. Plates 7 and 8 show mature diploid and haploid plants.

The haploid flower buds and heads were smaller than diploid ones (fig. 1). In a few instances only short rudimentary anther tubes were developed. The pollen was not pushed out of the anther tube by the elongating pistil at maturity as normally occurs, consequently the usual way of obtaining pollen for examination—dusting an albumen-coated slide with an opened flower head—failed on this account with haploid heads. It was necessary to remove the anther tubes and slit them open to free the pollen. The grains thus obtained were scant in number and the proportion of normally developed ones varied noticeably from floret to floret but it was always very small. The abnormal ones were small and faintly, or not at all, stained with aceto-carmin.

In the preliminary report it was stated that diploid areas were found in the roots of the haploid plants and the possibility was mentioned that parts of the plants above ground would be diploid and give rise to fertile branches. This possibility was realized. The first haploid to mature (28H.149-8) produced, early in its maturity, a tall branch bearing larger heads (pl. 8) and abundant pollen and, later, numerous achenes. Examination of the pollen mother cells (PMC's) revealed the diploid chromosome complex (below). It was presumed that the branch was wholly diploid until a haploid head appeared on it. The branch was possibly a diploid-haploid periclinal chimera, which would account for its larger size and the diploid nature of the reproductive organs. By a reorganization of tissue the haploid head could have been produced. This supposition is supported by the writer's impression (no measurements were made) that heads on a branch which appeared later were noticeably larger than those on the supposed chimeral branch. This later branch which arose from the same region of the crown produced only large heads with abundant pollen and more numerous achenes per head and was most probably wholly diploid.

Another haploid (29.036-9) produced three branches from one side of the crown, two of which had only diploid heads and the other had diploid, haploid, and chimeral diploid-haploid heads. This plant, grown in the unfavorable winter season, was attacked by mealy bugs and died before any achenes could be produced on the diploid heads. Two other haploid plants (28H.88-8 and 28H.54-19) produced chi-

meral diploid-haploid heads. Figure 2 shows haploid and diploid heads from plant 28H.149-8 and a chimera head from 28H.88-8. Plant 28X.20-2 showed no evidence of diploidy above ground. Plant 28H.50-46 died before maturity. The classification of heads as diploid or haploid after the first large heads were shown to be diploid, with respect to PMC's at least, was made on size alone. No achenes were produced on any of the haploid heads nor did reciprocal crosses

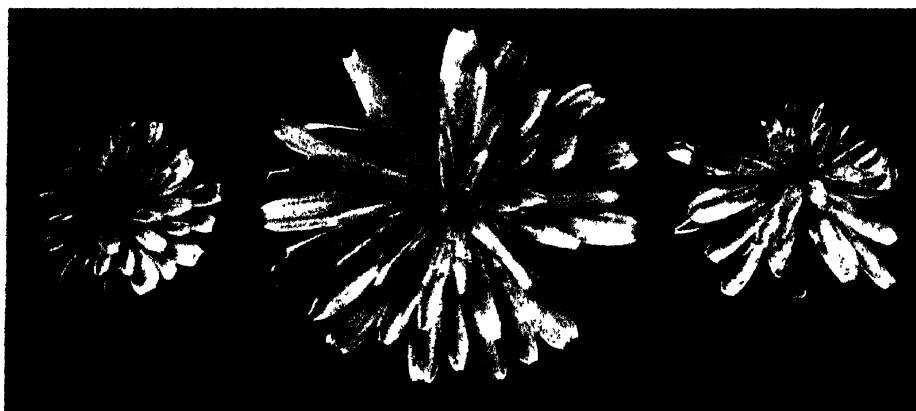


Fig. 2. Haploid and diploid heads from one haploid *Crepis capillaris* plant (28H.149-8) and a chimera head from another (28H.88-8). Twice natural size.

with normal *C. capillaris* give any achenes. Possibly more extensive trials would have resulted in success, for there was, as noted above, a small amount of apparently good pollen. However, 130 well formed achenes were obtained from the chimera(?) and diploid branches of plant 28H.149-8 which had been protected by a cage. The nature of *Crepis* pollen practically ensures selfing under such circumstances.

Since the diploid tissue arose from haploid, the pairs of chromosomes should be identical (barring mutation), and the progeny would be expected to be uniformly homozygous. In accord with expectation, the progeny were very uniform.

### CYTOLOGICAL METHODS

Root tips were fixed in Navashin's chromacetic-formalin, flower buds in the same solution preceded by treatment for several minutes with Carnoy's solution and imbedding followed in the usual manner. Other flower buds were fixed in Carnoy's solution alone, run into 70 per cent alcohol and there kept until ready for use, and examined in aceto-carmin (technique, Hollingshead 1930a). Both methods gave good pictures of meiotic chromosomes but the latter caused more cyto-



plasmic shrinkage. PMC's from the paraffin material were smaller than those in aceto-carmin as shown by the figures. Haidenhain's haematoxylin was used for both root tips and PMC's and iodine-gentian-violet, too, proved to be a useful PMC stain. When the latter was used it was found better to lengthen the time in the stain to an hour or so. Otherwise J. Clausen's schedule (Babcock and Clausen, 1929) was followed.

The drawings were made with the help of a camera lucida using a Zeiss 90X apochromatic 1.3 objective and a 20X compensating eyepiece giving a magnification of approximately 3000 and were reduced one-third in reproduction. Except where otherwise stated they are from aceto-carmin material.

### CYTOLOGICAL OBSERVATIONS

Diploid and haploid chromosome complexes from root tips are shown in figure 3. One member of each pair of chromosomes can be readily distinguished in the haploid complex. They behave normally in division. Similar complexes were observed in the walls of the ovaries of the haploid plants. The possibility that the chromosomes in haploids are smaller than those in diploids was mentioned in the preliminary note (1928). Further observations on this point were unconvincing.



Fig. 3. Somatic metaphases from root tips of diploid and haploid *Crepis capillaris* plants.

As stated above, diploid cells and even wholly diploid root tips were found in the haploid plants. The number of root tips examined from each plant, the number in which some diploid metaphases were found, and the number in which only diploid metaphases were seen, is given in table 1. Of the 110 root tips of all plants examined, 28 showed at least one diploid metaphase in otherwise haploid tissue, and in 42 root tips only diploid metaphases were seen. Since the number of diploid metaphases observed in a single root tip varied from one to many, it is probable that some of the roots in which only haploid metaphases were seen actually had some diploid cells at a stage other

than metaphase. The diploid metaphases were usually to be found in one particular area in each of a series of many sections, but occasionally they were found in two such portions of the root tip. In some cases the diploid portion included all of the different root tissues. No divisions were seen which showed how the doubling of the chromosomes took place. Diploid cells were generally considerably larger than haploid ones. One instance of a group of tetraploid cells in an otherwise diploid root tip, and another of a wholly tetraploid root tip were observed.

TABLE 1

## THE FREQUENCY OF DIPLOIDY IN ROOT TIPS OF HAPLOID PLANTS

| Plant Number | Number of root tips examined | Number with diploid and haploid metaphases | Number with only diploid metaphases |
|--------------|------------------------------|--|-------------------------------------|
| 29.036-9     | 17                           | 8  | 0                                   |
| 28H.50-46    | 14                           | 2  | 8*                                  |
| 28H.54-19    | 19                           | 3  | 6                                   |
| 28H.149-8    | 24                           | 10   | 2                                   |
| 28H.88-8     | 36                           | 5  | 26                                  |
| Total        | 110                          | 28   | 42                                  |

\*One tip contained a group of tetraploid cells.

The meiotic material studied has included only stages from diaphase to tetrad formation. The writer is not unmindful of the value of a study of the prophase of these plants with three chromosomes but lack of time has prevented her from undertaking it.

Meiotic divisions of diploid *C. capillaris* were described in a recent paper (Hollingshead, 1930a). A single diploid metaphase (fig. 4a) is shown here for comparison with the haploid. Bivalents vary considerably in shape from cell to cell, but it is usually easy to distinguish them from univalents, which were shown to occur rather frequently in some plants of this species.

The outstanding feature of the meiotic behavior of the haploid plants is its variability and irregularity. As was expected, three single chromosomes appear at diaphase (fig. 4b) contracting as this stage proceeds, to more or less spherical bodies (fig. 4c), often to be distinguished from the nucleolus only by their darker staining capacity.

The disappearance of the nuclear membrane is usually closely followed by that of the nucleolus, and the chromosomes lie free in the cytoplasm. Spindle fibers appear, but only in extremely rare cases is a metaphase plate formed. Chance appears to determine the distribution of the three chromosomes which commonly move to the

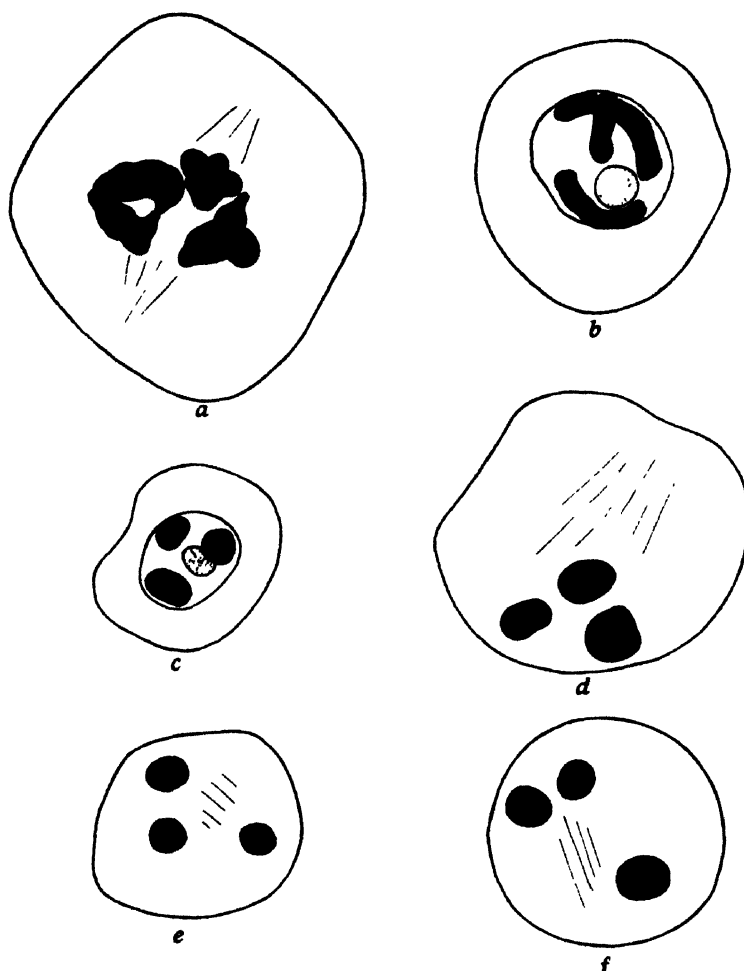


Fig. 4. *a*, diploid *Crepis capillaris*, 1M with three bivalents; *b-f*, haploid, heterotypic division; *b*, early diaphase, *c*, late diaphase, paraffin material, *d-f*, illustrating random segregation of univalents.

poles without division, still retaining their spherical shape (fig. 4, *d-f*). One occasionally lies outside the spindle and may fail to reach either pole.

In many cases, however, division of the chromosomes occurs, still without the formation of a metaphase plate. This division may be initiated, as shown by the elongation and constriction of the chromosome, and rarely is even completed at late diaphase (fig. 5*a*). As this

and later figures show, division of the chromosomes within a cell does not always proceed synchronously. Usually, however, the division begins later, and the chromosomes in the shape of attached twin spheres pass irregularly to the poles (fig. 5, *b*, *c*) or one may be left out of the spindle and fail to reach a pole, giving rise to three iso-

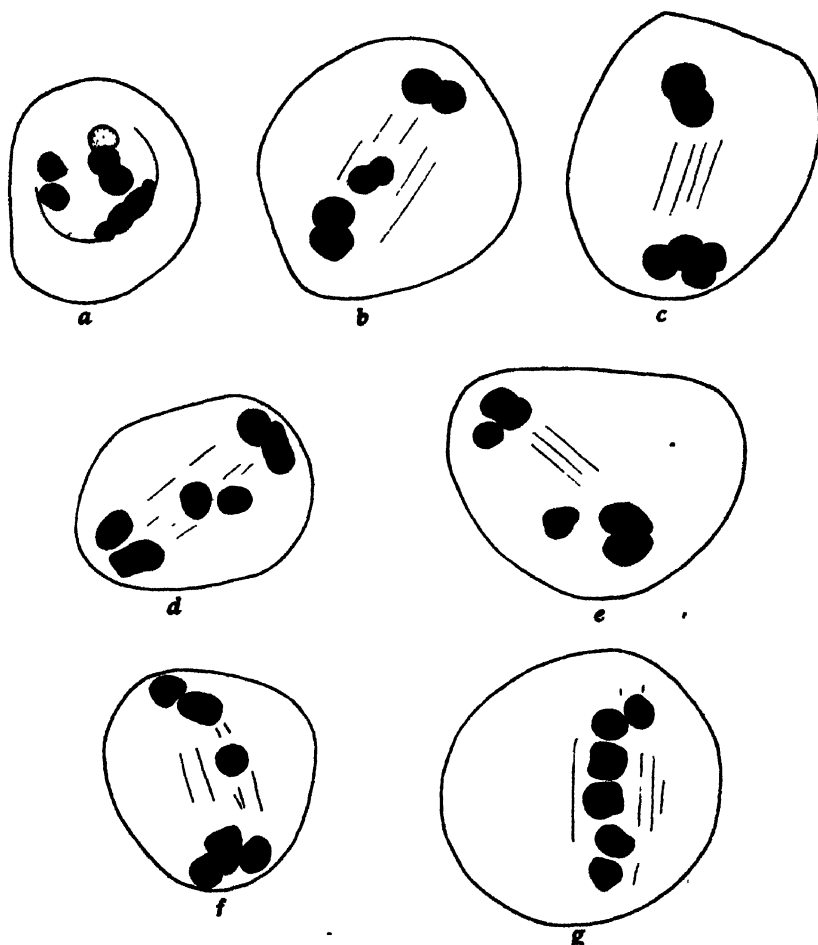


Fig. 5. Univalent division in haploid *Crepis capillaris*.  
*a*, late diaphase, *b-g*, 1M or 1A.

lated, partly divided chromosomes at late anaphase. Complete division of some or all of the chromosomes frequently occurs, but only in a small proportion of cases do the daughter halves separate far or pass to different poles (fig. 5, *d-g*); usually they remain close together and are incorporated in the same nucleus. The positions assumed by these combinations of divided and undivided chromosomes are so many and varied that it is impossible to illustrate them all. Figure 5, *d* and *g*, shows cells in which each chromosome has completed its

division, but the daughter halves have remained close together. Figure 5e shows one undivided, one almost divided, and one completely divided chromosome whose halves are well separated. Quite commonly, whole or half-chromosomes fail to reach either pole. These processes give rise to telophase nuclei varying greatly in chromosome number and constitution.

As implied above, the three chromosomes rarely do line up on a metaphase plate and divide normally (fig. 6 *a, b*). In shape they are

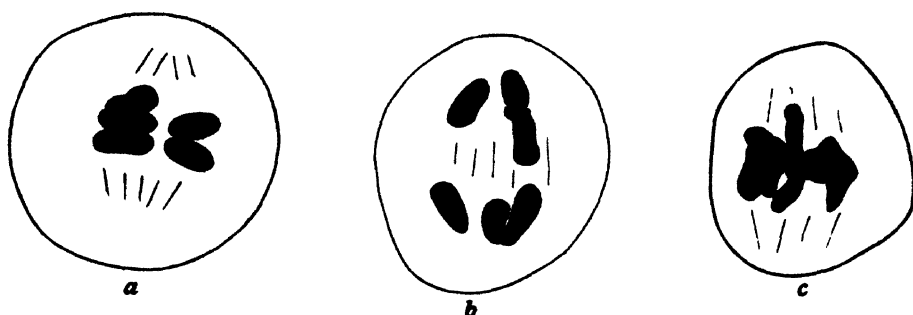


Fig. 6. Haploid *Crepis capillaris*. *a, b*, division and separation of three univalents; *c*, three bivalents at 1 M.

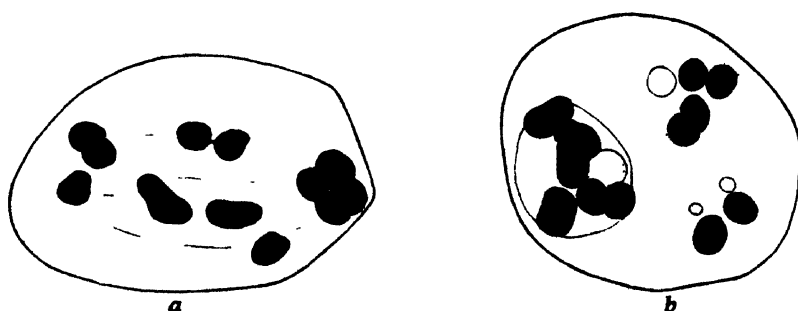


Fig. 7. Haploid *Crepis capillaris*. *a*, anaphase with eight single and two partly divided chromosomes; *b*, early telophase with twelve chromosomes.

typical dividing heterotypic univalents (cf. fig. 13c). In one slide, too, a few cells with three normal bivalents (fig. 6c) were seen. These probably arose from diploid cells in the lineage of the PMC's and normal meiotic behavior would presumably ensue. These two processes would be expected to give rise to gametes of normal chromosome constitution.

Several cells were observed with more than six chromosomes. One such is shown in figure 7a, where eight more or less spherical and two elongated constricted chromosomes are to be seen. Most of the chromosomes are in pairs and have evidently just finished dividing, and the two large ones are undergoing division. Counting the two divid-

ing chromosomes as four, the total of twelve suggests two successive divisions of three, or a single division of six univalents, the cause of either of which is obscure. A later stage—very early telophase—resulting from such a complex, is shown in figure 7*b*. Twelve chromosomes in groups of various numbers are to be seen and nucleoli and nuclear membranes are being differentiated. Other cells with twelve chromosomes in two or three groups at telophase were also observed.

One instance of two metaphase plates in a single PMC, each containing three bivalents, was observed (fig. 8). No explanation of this unusual occurrence is ventured.

The number and constitution of telophase nuclei are readily studied at this stage, for contrary to the normal behavior in this



Fig. 8. Haploid *Crepis capillaris*. A cell containing two 1M plates each consisting of three bivalents.

genus, in most cases the chromosomes are clearly outlined and often can be distinguished from each other by their relative sizes in the telophase nuclei. Occasionally, however, a group of telophases is observed in which the chromosome outlines are not clear and the reforming nuclei resemble masses of chromatin. Telophase usually begins with the appearance of a clear area in the cytoplasm surrounding a clearly outlined chromosome or group of such chromosomes. An instance of an interesting and rare stage, the appearance of a faint nucleolus near each group of chromosomes before the appearance of the nuclear membrane, was observed, and is shown in figure 9*a*. Occasionally the nuclear membrane is formed around a chromosome or group of chromosomes without including a nucleolus. The telophase chromosomes elongate and commonly total six in number (fig. 9, *b-f*). Chromosomes whose divisions have been initiated but which have not divided on the spindle, complete their division at telophase within the nuclear membrane. It has not been ascertained whether chromosomes which showed no sign of division on the spindle divide at telophase, but it is possible that they do so, for among the many

telophase cells observed in which chromosomes could be distinguished, all had more than three and most had six chromosomes. A few instances of more than six chromosomes at this stage were observed (one with eight is shown in figure 9g). This could have arisen from non-disjunction in a previous division although no evidence of such an occurrence was seen in somatic cells. Telophase cells with twelve chromosomes have already been discussed.

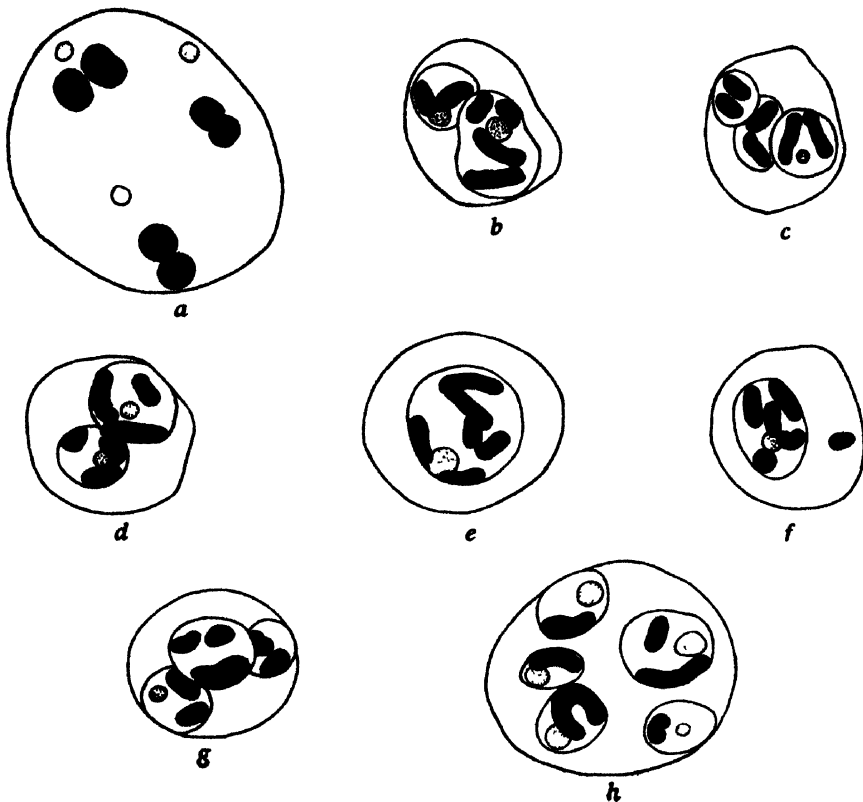


Fig. 9. Haploid *Crepis capillaris*; heterotypic telophases; b-g, paraffin material.

The previous distribution of the chromosomes determines the number and constitution of the telophase nuclei. The number observed varied from one (fig. 9e) to five (fig. 9h), two (fig. 9b, d), and three (fig. 9c) being most common. Since chromosomes complete their division at this stage they commonly occur in pairs (fig. 9, b-e). However, an unpaired chromosome in a nucleus, the result of separation of daughter halves to different poles at anaphase, is to be seen occasionally (fig. 9d). These figures illustrate some of the many varied telophase combinations observed.

The telophase chromosomes continue to elongate (fig. 10, *a*, *b*) and the nucleus gradually passes into a typical interphase condition. Before this change is completed, furrowing begins to take place (fig. 10*c*) thus initiating microspore formation. No second meiotic divisions have ever been seen and the nature of the young microspore groups which are commonly diads and triads instead of tetrads, supports the conclusion that ordinarily no homeotypic division occurs.

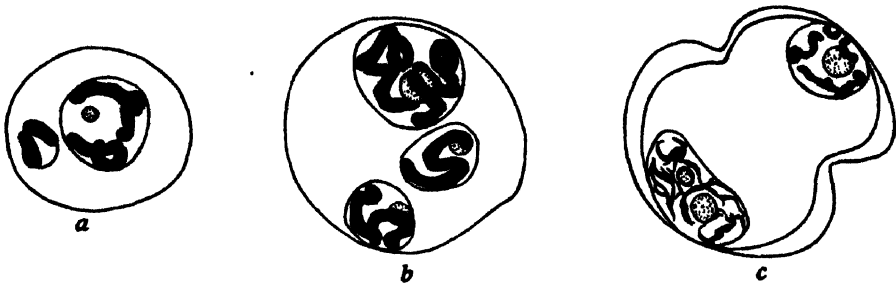


Fig. 10. Haploid *Crepis capillaris*. *a*, *b*, elongating chromosomes at 1T; *c*, furrowing. *a*, paraffin material.

TABLE 2

THE FREQUENCY OF TETRADS, TRIADS, ETC., IN HAPLOID PLANTS

| Plant No. | Tetrad | Triad | Diad | Monad |
|-----------|--------|-------|------|-------|
| 28H.149-8 | 8      | 23    | 52   | 5     |
| 28H.54-19 | 10     | 15    | 69   | 5     |
| 28X20-2   | 1      | 19    | 74   | 2     |
| Total     | 19     | 57    | 195  | 12    |

Table 2 shows the frequency with which tetrads, triads, diads, and monads were observed in smears from three buds. Diads are most frequent, triads are fairly common, monads, and tetrads are infrequent and only one instance of a pentad (in another smear) was seen. Young microspores frequently contain two nuclei. As would be expected from the variation in chromosome number in telophase nuclei, the young microspores in most groups are very different in size. Indeed many of the microspore groups called tetrads or triads would be better described as triads or diads with one or two microcytes. Diad cells are usually markedly different in size. Figure 11 illustrates a few of the many kinds of microspore groups observed.



It is obvious that very few of the microspores will contain a normal haploid chromosome complex. Diads, usually the result of non-reduction, are here commonly the result of a suppression of the homeotypic division and contain chromosome complexes varying widely in

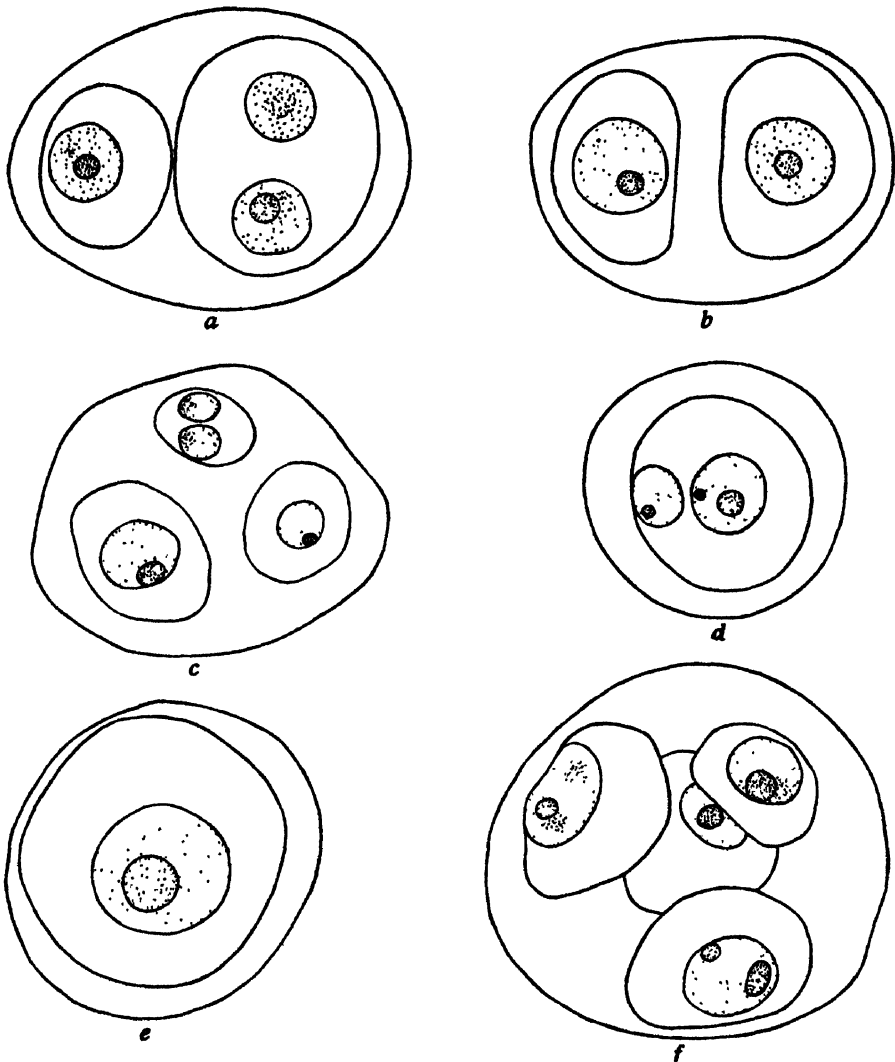


Fig. 11. Haploid *Crepis capillaris*. Representative microspore groups.

number and constitution. The diad which presumably results from division of all the chromosomes, and the tetrad which presumably arises from a diploid PMC, are probably the only ones which produce normal haploid gametes. As noted above, normal pollen grains were very rare. The monad presumably contains the diploid chromosome complex and would be expected to give rise to a diploid pollen grain.

## MEIOSIS IN DIPLOID TISSUE OF A HAPLOID

Several buds of the chimeral(?) branch on plant 28H.149-8 which bore large heads were fixed and, as stated above, showed the diploid chromosome complex (fig. 12a). The members of each of the three pairs of chromosomes were presumably completely homologous (bar-ring mutation), yet a high degree of non-conjunction occurred. Figure 12, b-d, shows first metaphases illustrating non-conjunction of one, two, and three pairs respectively. This failure to pair could be seen in late diaphase as illustrated in figure 13, a, b, which shows two and

TABLE 3

THE FREQUENCY OF FIRST METAPHASES WITH VARIOUS COMBINATIONS OF  
BIVALENTS AND UNIVALENTS IN THE DIPLOID PMC'S FROM BUDS ON  
A CHIMERAL(?) BRANCH OF THE HAPLOID PLANT 28H.149-8

| 3" | 2"-4' | 1"-4' | 6' |
|----|-------|-------|----|
| 42 | 51    | 22    | 6  |
| 43 | 48    | 19    | 2  |

six unpaired chromosomes respectively. Table 3 shows the frequency of first metaphase cells with three, two, one, and no bivalents respectively in two different buds. The amount of non-conjunction is comparable with that found in certain diploid plants of the *capillaris* X strain (Hollingshead, 1930b) which is the strain from which this haploid was derived.

The univalents either segregated without division or divided (fig. 13c), but no particular study of the later stages in microspore formation was undertaken except to note the frequent occurrence of micronuclei and microcytes in the tetrads. It is obvious that gametes with abnormal chromosome constitution would be formed frequently, and that these gave rise in some cases to inviable pollen grains, is indicated by the one pollen count made which showed 173 small and unstained grains in a total of 580, or nearly 30 per cent bad pollen. As stated above, the progeny of the branch from which these buds were obtained and that from a later diploid one, were normal and remarkably uniform. Probably no abnormal gametes functioned.

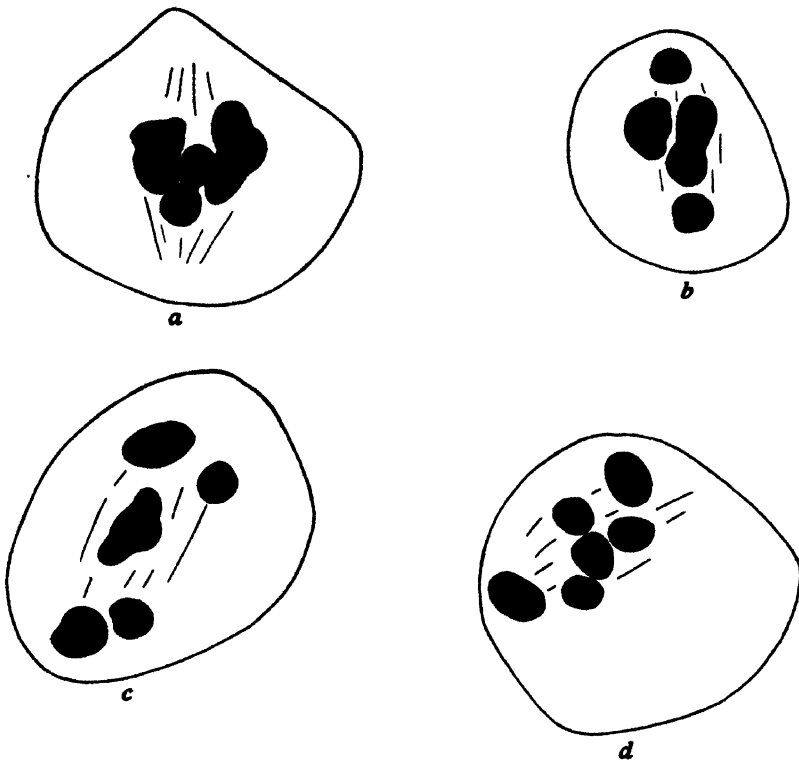


Fig. 12. 1 M in diploid tissue of haploid *Crepis capillaris*. a, normal, b-d, non-conjunction in one, two, and three pairs.

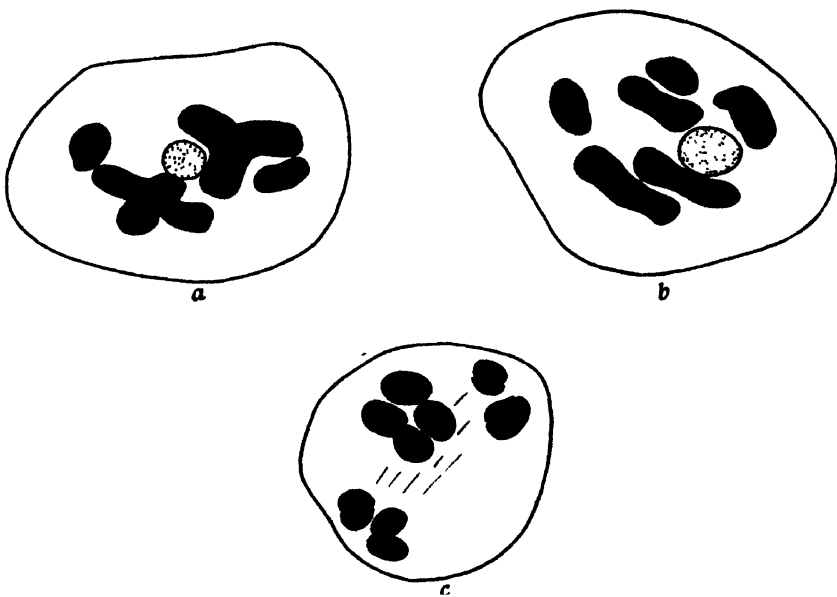


Fig. 13. Diploid tissue of haploid *Crepis capillaris*. a, b, non-conjunction of one and three pairs at diaphase; c, division of two univalents at heterotypic anaphase.

## DISCUSSION

The list of reported instances of haploid sporophytes in Angiosperms includes at the time of writing *Datura stramonium*, *Nicotiana tabacum*, *N. glutinosa*, *N. Langsdorffii*, *Triticum compactum*, *Matthiola incana* (a disomic haploid), *Solanum nigrum*, *S. lycopersicum*, *Oenothera franciscana*, *O. rubricalyx*, *O. Hookeri*, *O. argillicola*, and *Crepis capillaris*. It has not yet been possible to secure a paper by Khristov (1930) on "A haploid tobacco plant." Some of the papers dealing with these haploids are specifically referred to below while others are cited only in the bibliography. As the literature on haploid plants has been reviewed recently in detail by Gates and Goodwin (1930) this discussion will be confined to a consideration of points in which the *Crepis* haploids resemble or differ from others previously described.

With the exception of haploid plants of *Nicotiana tabacum* (Clausen and Lammerts, 1929) and of *N. Langsdorffii* (Kostoff, 1929) each of which developed from a sperm nucleus thus becoming the first authentic cases of haploid merogony in higher plants, the origin of most of the haploids has been attributed to the development of unfertilized egg cells. The *Crepis* haploids doubtless arose in this same way. Foreign pollen in most cases, but cold in the case of some *Datura* haploids, probably acted as a stimulus for development, but in others, where the haploids arose in pure-line cultures (*Nicotiana glutinosa* and *Oenothera franciscana*), no stimulating factor is known. In the last two cases it is conceivable but less probable, that sperm nuclei developed into haploid plants. In the preliminary note it was stated that cold weather prevailed at the time of origin of the first two *Crepis* haploids, and it was suggested that this circumstance might have been a contributing factor in their origin. The occurrence of later haploids which developed under varying weather conditions seems to eliminate cold as a stimulating factor, and leaves the foreign pollen of *C. tectorum* and *C. setosa* as the probable stimulus to development.

As in *Crepis*, the haploid nature of a plant having been once established, it is usually possible to identify others of the same species by their similar morphology. They are frequently described as reduced replicas of diploids differing sometimes in minor characters, e.g.,

details of leaf shape in *Nicotiana tabacum* and *Oenothera franciscana* and flower color in *Nicotiana glutinosa*. However, considerable variation in degree of resemblance to diploids occurs, for the *Triticum* haploid could be distinguished from diploids only by its sterility, and the *Oenothera rubricalyx* haploid was described as being very much dwarfed. *Crepis* haploids were smaller than diploid plants and differed noticeably from them in other characters, particularly in leaf shape. In sterility the *Crepis* haploids resemble those of other genera, which were highly or completely sterile.

Diploid somatic cells have been found in haploid *Nicotiana* plants (Ruttle, 1928, Kostoff, 1929), and in the *Solanum* haploid (Lindstrom, 1929), and cells with apparently fusing nuclei were seen in somatic tissue of the *Triticum compactum* haploid (Gaines and Aase, 1926). The fusion of daughter nuclei not separated by a cell wall has been the most popular suggested explanation for the doubling of chromosomes in somatic cells. Why this doubling should occur much more frequently in haploid than in diploid tissue, as shown by Gaines and Aase (1926) in *Triticum*, by Ruttle (1928) in *Nicotiana*, and by the present work on *Crepis* haploids is unknown, but there undoubtedly exists in these haploids a distinct tendency toward diploidy.

Ruttle and Lindstrom sought in vain for diploid branches on their many *S. lycopersicum* and *N. tabacum* haploid cuttings, but one of the original *Datura* haploids propagated by cuttings, produced a branch characterized by a small proportion of aborted pollen grains and large capsules (Davenport, 1927). Dr. A. D. Bergner has kindly given further unpublished data in this connection. That this branch was diploid was shown by a chromosome count of both PMC's and root tip cells, cuttings from the branch having been rooted in sand. Dr. Bergner has also investigated a haploid-diploid periclinal chimera branch of this *Datura* haploid plant which had diploid root tips and haploid PMC's. The diploid and chimera branches or heads on the *Crepis* haploids indicated that doubling of chromosomes in the parts above ground took place rather frequently.

In meiotic behavior, the *Crepis* haploids differ in several respects from most others hitherto described. They are the most variable in behavior and exhibit hitherto unreported features in the occasional division of univalent chromosomes at diaphase, and the omission of the homeotypic division. Initiation and completion of univalent division at diaphase, while not described hitherto in haploids, has been

observed in *Crepis capillaris*-*C. aspera*  $F_1$  hybrids with seven unpaired chromosomes (Navashin, 1927). The division of some of the univalents at first anaphase while others were segregating undivided, is another feature in which the *Crepis* haploids resemble Navashin's *capillaris-aspera* hybrids. Among haploids *Nicotiana tabacum* (Chipman and Goodspeed, 1927) and *Matthiola incana* (Lesley and Frost, 1928) exhibit this behavior. The failure of halves of divided univalents to separate to opposite poles has been observed in some hybrids with many univalents, as in wheat-rye hybrids (Thompson, 1926), and this was observed in *Triticum compactum* and *Nicotiana tabacum* haploids by Gaines and Aase (1926), and Clausen and Lammerts (1929). Blakeslee, Morrison and Avery (1927) have shown that *Datura* haploid plants throw a markedly higher percentage of trisomics than do diploids. They attribute it to a possible non-disjunction in pre- or post-meiotic divisions but it might be that it is the result of the failure of a pair of univalent halves to disjoin in meiosis.

In lack of pairing and in prevalence of random segregation of univalents at the first division, the *Crepis* haploids resemble most of the others described. Of all haploids only *Solanum nigrum* is known to exhibit true pairing (Jorgensen, 1928) and it was only in the *Matthiola* haploid that the usual mode of behavior was, a division of all the univalents following the formation of a regular plate at first metaphase.

The occurrence of divided chromosomes at heterotypic telophase after the formation of a nuclear membrane, is the normal condition in many plants. In normal *Crepis capillaris*, however, after the formation of the nuclear membrane the individual chromosomes cannot usually be distinguished. In the haploid plants telophases in which paired and single chromosomes lay distinct and clear within the nuclear membrane were the more striking by their contrast with telophases in normal plants of this species. This stage, here interpreted as heterotypic telophase, bears a marked resemblance to that described as homeotypic prophase in *C. capillaris-aspera* hybrids by Navashin. In these plants, as in the haploids, halves of chromosomes which had divided and had separated during the first anaphase lay singly, while chromosomes which had segregated without division, had divided and lay in pairs within the nuclear membranes.

Considerable attention has been paid to the question whether this stage in the haploids could be homeotypic prophase and the possibility should not be overlooked completely. According to the latter inter-

pretation the occasional groups of nuclei which appear to form directly from anaphase chromosomes as their outlines grow increasingly indistinct might be considered typical telophases. The others, in which the chromosomes are clearly seen, would be homeotypic prophases. There were no homeotypic metaphases, so, according to this interpretation, the prophase nuclei would pass immediately into the interphase condition again and furrowing ensue. Although no positive evidence has been secured to disprove this interpretation, because of the simpler nature of the process, and the evidence from the seriation of stages within anther locules, the writer decidedly favors the interpretation given in the description of meiotic behavior. It should be noted however, that evidence from seriation of stages must be accepted with some reservation, for while all the PMC's in one locule are usually at about the same stage occasionally groups of cells at very different stages may lie in the same locule. Even if homeotypic divisions are initiated, however, and proceed to prophases they are really omitted for no further separation of chromosome halves takes place and the nuclei resulting from the heterotypic division persist unchanged.

There is no obvious cause for this omission of the homeotypic division. It has been shown that the chromosomes at heterotypic telophase are usually divided and apparently ready for the next division. Furrowing takes place, however, and young microspores are formed forthwith. This gives rise, as pointed out earlier, to a preponderance of diads, whose chromosome constitution, contrary to the general rule, is far from that of a haploid complex. In the *Nicotiana Langsdorffii* haploid the homeotypic metaphase was sometimes omitted (Kostoff, 1929). The chromosomes often spread out over the entire spindle at the heterotypic division and underwent interkinesis together. After such an interkinesis sometimes the chromosomes did not become organized into a normal equatorial plate. They divided, however, and formed a monad.

The small amount of apparently good pollen and the sterility of these haploid plants are easily understood in the light of their meiotic behavior. Division and separation of all the univalents to different nuclei, or the occurrence and normal behavior of three bivalents (processes which would probably give rise to gametes with the haploid chromosome complex), were rare occurrences. Even the few apparently normal male gametes had little chance of effecting fertilization for, as was pointed out, pollen was not extruded from the anther tubes as in normal florets. Possibly seeds could have been obtained

with more extensive hand pollination but it was not thought worth while to prolong these attempts.

The relatively large amount of non-conjunction in diploid tissue of a haploid plant is of great theoretical interest. Meiotic pairing is usually assumed to be evidence of chromosome homology, and when it frequently fails to take place the chromosomes are often considered to be weakly homologous. It is well known of course that occasionally two chromosomes which normally pair, fail to do so, but in this instance we have frequent non-conjunction between completely homologous chromosomes. This is clear evidence that complete homology alone does not necessarily result in bivalent formation. Diploid plants of this strain of *C. capillaris* frequently exhibit non-conjunction (Hollingshead, 1930a) in varying amounts. It is clear now that this is not necessarily due to heterozygosity. Possibly further studies on this strain might throw some light on what other factors are involved in the non-conjunction of chromosomes in a normal diploid complex.

## SUMMARY

Five *C. capillaris* haploid plants were found in populations of *C. capillaris-tectorum* F<sub>1</sub> hybrids numbering over three thousand plants. A sixth haploid was one of two plants resulting from a *C. capillaris*-*C. setosa* cross. They doubtless resulted from the parthenogenetic development of *capillaris* egg cells.

The haploids resembled reduced diploids but differed noticeably from diploids in leaf shape and habit of growth.

Root tips usually showed the haploid chromosome complex of three individually different chromosomes but parts of some root tips in each haploid were diploid and some root tips were wholly diploid. A few parts of most of the plants above ground were also diploid, giving rise to diploid and chimera heads and branches.

The haploid portions of the plants were sterile, but achenes were obtained from diploid parts of one haploid plant. The progeny, presumably completely homozygous, were remarkably uniform in appearance.

In meiotic behavior (PMC's) the haploids were irregular and variable. They resembled other haploids previously described in the occurrence of a random segregation of univalents at the heterotypic



division, and of a rare division of all univalents followed by separation of the daughter halves to different poles, or "non-reduction." New or unusual features were (1) the occasional division of univalents at diaphase, (2) the frequent division of univalents but the inclusion of most pairs of daughter halves in the same nucleus, and (3) the omission of the homeotypic division. As a result microspores of normal chromosome constitution were rarely formed and very little good pollen was produced.

In diploid tissue on a haploid plant three bivalents were formed in many PMC's but non-conjunction of one or more chromosome pairs was a frequent occurrence. This lack of pairing between presumably completely homologous chromosomes is of great theoretical interest, for it shows that complete homology does not necessarily result in bivalent formation at meiosis.

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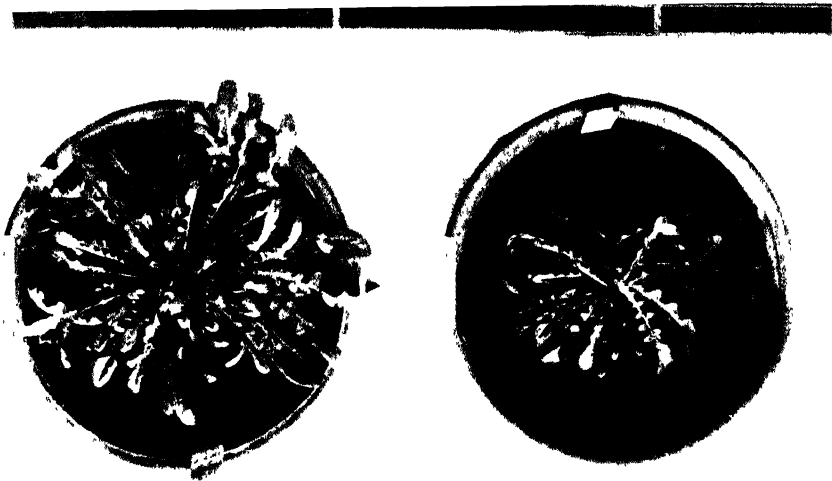
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**PLATE 6**

***Crepis capillaris.***

***a*, Haploid plants at the rosette stage.**

***b*, Haploid and diploid plants of the same age.**



a



b

**PLATE 7**

*Crepis capillaris.*

Diploid plant at late maturity.



PLATE 8

*Crepis capillaris.*

Haploid plant at early maturity showing the tall chimeral(?) branch with large heads.





**CYTOLOGICAL STUDIES OF  
FIVE INTERSPECIFIC HYBRIDS OF  
CREPIS LEONTODONTOIDES**

**BY**

**PRISCILLA AVERY**

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CYTOLOGICAL STUDIES OF  
FIVE INTERSPECIFIC HYBRIDS OF  
CREPIS LEONTODONTOIDES

BY  
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# CYTOLOGICAL STUDIES OF FIVE INTERSPECIFIC HYBRIDS OF *CREPIS LEONTODONTOIDES*

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## INTRODUCTION

Cytological investigations in *Crepis* have been primarily concerned with the somatic chromosomes, their low number and well marked individuality being particularly favorable for such studies. Less extensive investigations have been carried on in regard to meiotic phenomena in interspecific hybrids by Collins and Mann (1923), Navashin (1927), Babcock and Clausen (1929), and Hollingshead (1930a). The study of this phase of the chromosome cycle involves greater difficulties than that of the somatic and must often be inferior on the quantitative side to similar studies in other more easily handled genera. Nevertheless, certain apparently significant facts are very strikingly demonstrated in the meiotic as well as the somatic divisions of the hybrids of low-chromosomed species. It is therefore in illustration of these facts that the present account is given of cytological observations on five  $F_1$  hybrids in which a single species, *Crepis leontodontoides*, served as one parent in the cross.

It is a pleasure to acknowledge my indebtedness to Professor E. B. Babcock for advice and interest as well as for facilities which made possible the studies reported here. I am also indebted to Mr. C. W. Haney for three of the hybrids used in these investigations.

## MATERIAL AND METHODS

Two accessions (1682 and 1807) of seed from Italy were the source of the *C. leontodontoides* plants used in securing the hybrids concerned in the present study. The plants of the other species used were those belonging to strains grown in pure line in the Genetics gardens at the University of California (*C. tectorum*, 1498 and 1622; *C. capillaris* X-strain; *C. parviflora*, 1533; *C. marschalli*, 1532; *C. aurea*, 1636).

Root tips from young plants were used as material for the study of somatic chromosomes. These were killed and fixed in chrom-acetic formalin according to formula 1 given by Hollingshead and Babcock (1930, p. 3), and imbedded in paraffin in the usual way. Sections were cut  $8\mu$  thick and stained with Haidenhain's iron-alum haematoxylin.

For the study of meiotic behavior, PMC's were used, three different procedures being followed. In a few cases, and especially for tetrad counts, aceto-carminine smears of buds of the proper stage were used. Most frequently, however, the buds of approximately the right stage were fixed in Carnoy's fluid for 3–12 hours, rinsed in the higher alcohols, and stored in 70 per cent alcohol. These buds were then used for making smears with aceto-carminine as described by Hollingshead (1930a). One objection to this procedure is the large loss of PMC's in hybrids where material is apt to be scarce. In these cases some buds were fixed in chrom-acetic-formalin, or in Carnoy followed by chrom-acetic-formalin as described by Babcock and Clausen (1929). After imbedding, these buds were sectioned 10–12 $\mu$  in thickness and stained with either haematoxylin or gentian violet. All drawings were made from aceto-carminine smears, the sectioned material being used to supplement these in the study of meiotic behavior.

The somatic chromosomes were drawn at a magnification of 3400 and reduced one-fourth in reproduction. The meiotic phases were drawn at the same magnification (except figs. 5a and 5c,  $\times 3700$ ) and reduced one-half in reproduction.

#### THE SPECIES USED IN THE CROSSES AND THEIR $F_1$ HYBRIDS

The species hybrids used in the present study were made between *Crepis leontodontoides* and five other *Crepis* species belonging to three subgenera. *C. leontodontoides* has been placed in the subgenus *Eucrepis* because it has a spongy thickened mid-rib on the inner involucre bracts, and the achenes found in some forms are beakless. However, the very short beaks of the achenes in certain other forms have caused this species to be classified under *Barkhausia* in several works. In fact *C. leontodontoides* is really a border-line species with reference to the three large subgenera of *Crepis*, for the evidence herein reported certainly indicates a derivation in common with one or more species of *Catonia*. To the subgenus *Eucrepis* belong the species *C. capillaris*, *C. tectorum*, and *C. parviflora*, with all of which species  $F_1$  hybrids

with *C. leontodontoides* were obtained. *C. aurea* is the only species of the subgenus *Catonia* with which hybrids were secured, although extensive crosses were made with *C. tingitana*.  $F_1$  hybrids were also secured with the *Barkhausia* species *C. marschalli*.

In every case the  $F_1$  hybrids possessed features characteristic of each of the two parental species. In three cases the hybrid resembled one parent more closely than the other in its gross morphology.



Fig. 1. Rosette leaves of *Crepis leontodontoides*, *C. capillaris* and between them their  $F_1$  hybrid.

Thus the hybrids between *leontodontoides* and *aurea* and between *leontodontoides* and *marschalli* were closer to *leontodontoides* than to the other parent; while the  $F_1$  *leontodontoides*  $\times$  *parviflora* resembled *parviflora* more closely. In the other two cases,  $F_1$  *leontodontoides*  $\times$  *capillaris* and  $F_1$  *leontodontoides*  $\times$  *tectorum*, the hybrid was intermediate in habit and in all character expressions. This is well shown by a comparison of the rosette leaves of *C. leontodontoides*, *C. capillaris* and their  $F_1$  hybrid (fig. 1).

In the  $F_1$  hybrid between *C. leontodontoides* and *C. tectorum* a lethal Mendelian factor was operative, causing the death in the cotyledon stage of all the hybrids from four different *tectorum* plants, and of one-half the hybrids from two *tectorum* plants. In one case all



the hybrids from one *tectorum* plant were viable. The genetic analysis of the same lethal factor operating in the  $F_1$  hybrid between *C. tectorum* and *C. capillaris* has been made by Hollingshead (1930b).

A summary of the vigor, fertility, and morphological characters of these  $F_1$  hybrids as determined in the present investigation has been given by Babcock and Navashin (1930, pp. 59–63). Their possible significance, in connection with the cytological evidence presented here, for the determination of phylogenetic and taxonomic relationships has also been pointed out in this report (p. 62).

The group of hybrids thus available for study possessed the haploid chromosome complement of *C. leontodontoides* in combination with five different genomes belonging to *Crepis* species of various degrees of taxonomic relationship.

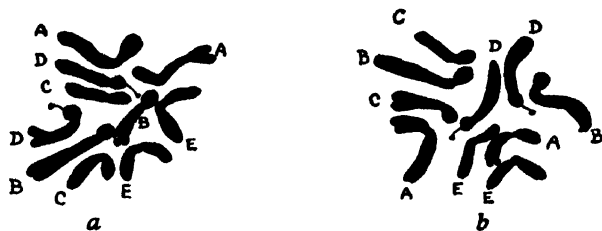


Fig. 2. Somatic metaphases of *C. leontodontoides*.

#### THE SOMATIC CHROMOSOME COMPLEMENT OF *C. LEONTODONTOIDES*

The characteristic morphology of the somatic chromosomes of *C. leontodontoides* was studied in considerable detail in order to facilitate their recognition in the chromosome complements of the hybrids, and make possible their comparison with chromosomes of other species.

In individuality the chromosomes of *C. leontodontoides* are less well marked than are those of most *Crepis* species with low chromosome number. This is largely due to the smaller size of the chromosomes and the lack of great differences in size within the complement. A study of many somatic plates showed, however, that each of the members of the five pairs can be distinguished, chiefly on the basis of the fiber attachment constriction. It was nevertheless difficult to find plates in which all ten chromosomes were in a position to show their characteristic morphologies (figs. 2a and 2b).

In the *leontodontoides* haploid set of five, three chromosomes differ little in total length, but they may be distinguished by the point of constriction where the spindle fiber is attached. Following Navashin's scheme, A has been used to designate one of the long chromosomes

of the set, having a submedian point of constriction about one-third of the distance from the proximal to the distal end, giving the chromosome a J-shape (fig. 2). A chromosome of about the same length, but with a subterminal constriction forming a head is designated B. Because of the position of the spindle fiber attachment, this chromosome often lies unbent in the plate and appears to be longer than the A-chromosome. When, however, it is bent, the B-chromosome is very similar to the A-chromosome in appearance and may be distinguished from it only if the subterminal constriction is evident. The third of the long chromosomes is slightly shorter than the other two, and is characterized by a median constriction which typically gives a V shape to this E-chromosome.

The D-chromosome is intermediate in length, but is only slightly shorter than the E-chromosome. It is, however, well marked because it bears a small but distinct satellite attached by a slender thread to the proximal end of the chromosome. The constriction is subterminal, forming a small head to which the satellite is attached, but this point of constriction is often less well marked than are those of the other chromosomes. Typically, the D-chromosome lies unbent and nearly straight in the plate, but it sometimes bends at a point other than that of constriction.

The shortest C-chromosome is distinctly shorter than any of the rest. It has a submedian constriction forming arms which hold about the same relation to each other as those of the A-chromosome, i.e., 2:1. It is the only chromosome which can be identified from size relations alone.

### $F_1$ *C. leontodontoides* $\times$ *C. tectorum*

#### THE SOMATIC CHROMOSOMES

None of the five chromosomes of *C. leontodontoides* resembles any of the four chromosomes of *C. tectorum* in somatic figures. The difference between the chromosomes of the two species is striking in the somatic cells of the hybrid. Here the *leontodontoides* chromosomes seem small in comparison to those of *tectorum*, and also the slight differences in length within the *leontodontoides* complement as compared with the greater differences in *tectorum* are evident (fig. 3).

The total length of the five *leontodontoides* chromosomes is less than three-fourths the length of the four *tectorum* chromosomes as measured in the same somatic cells of the hybrid. Each of the four *tectorum* chromosomes is easily recognized, but often the slight differ-

ences between the *leontodontoides* chromosomes are not evident, especially since the D-chromosome loses its satellite in the hybrid. The D-chromosome of *tectorum* retains its satellite unaltered, but in no case was a satellite found on the *leontodontoides* chromosome, a phenomenon (amphiplastie) which seems to be characteristic of certain interspecific hybrids as first described by Navashin (1928). Figure 3 shows a somatic plate in which the differences between the two haploid sets are evident, and each of the chromosomes from the two parents may be identified.



Fig. 3. Somatic metaphase of  $F_1$  *C. leontodontoides-tectorum*.

### MEIOSIS

With such morphological differences between the parental chromosomes, there is little reason to expect conjugation of the chromosomes of the two species at the meiotic divisions in the hybrid. Accordingly, at diakinesis and metaphase of the first meiotic division the nine chromosomes of the hybrid are frequently unpaired. In figure 4a a diakinesis stage shows nine unpaired units of which the four larger *tectorum* chromosomes are distinct from the five smaller and rounder *leontodontoides* chromosomes. Occasionally a stage immediately following the disappearance of the nuclear membrane shows the nine units arranged in a semicircular fashion, suggesting a telosynaptic arrangement (fig. 4b). Where no pairing has occurred up to diakinesis, there is apparently little tendency for the formation of a definite first metaphase plate. Thus a scattering distribution of the nine unpaired chromosomes (fig. 4c) seems to follow directly upon such a late diakinesis as that shown in figure 4b. Rarely a I-M is seen in which all the nine units are rather definitely arranged in an equatorial plate, and here the four large *tectorum* chromosomes are distinct from the five small *leontodontoides* chromosomes (figs. 4d and 4e). Only instances of random distribution of the unpaired chromo-

somes to the two poles were seen, with occasional laggards which sometimes divide. It seems probable, however, that here, as in some of the other hybrids, all nine chromosomes occasionally divide in the first division, giving rise to dyads and diploid gametes. At the tetrad stage about 7 per cent dyads are present, the proportion varying from 5.2 to 8.9 per cent.

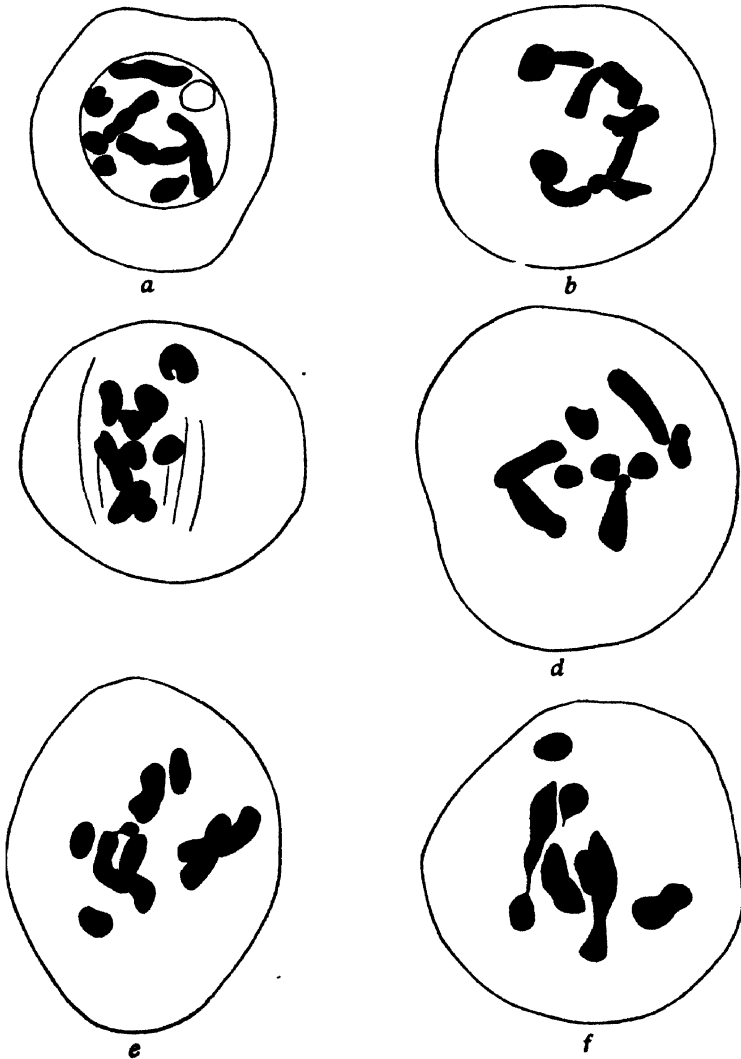


Fig. 4. Meiosis in  $F_1$  *C. leontodontoides-tectorum*.

*a*, diakinesis, no bivalents; *b*, early I-M, no bivalents; *c*, I-A distribution following no pairing in I; *d*, and *e*, I-M, no bivalents, showing four large *tectorum* and five smaller *leontodontoides* chromosomes; *f*, I-M, showing one bivalent, one loosely conjugated unequal pair, and five unpaired chromosomes.

Although the behavior of the chromosomes in meiosis just described was rather frequently seen in the hybrid, it was far from being the

characteristic behavior at the reduction division. All degrees of association between the chromosomes of the two sets were observed, from  $1_{II} + 7_I$  to  $4_{II} + 1_I$ . Sometimes "loose" pairing of two chromosomes added to the irregular appearance of the first metaphase. A tabulation of 54 PMC's in diakinesis and I-M in which the number of pairs and singles was readily interpreted (table 1) showed that  $2_{II} + 5_I$  were most frequent,  $9_I$  and  $4_{II} + 1_I$  about equally frequent, and  $1_{II} + 7_I$  and  $3_{II} + 3_I$  least, although only slightly less, frequent. Examples of these types of pairing are shown in figures 4f and 5. In figure 4f there is  $1_{II} + 7_I$ , with two unequal univalents showing loose association; in figure 5a,  $2_{II} + 5_I$ ; in figure 5c, a condition intermediate between the last two is shown, there being  $1_{II} + 1$  loose  $+ 5_I$ ; in figure 5b there are  $3_{II} + 3_I$ ; in figure 5d a diakinesis shows  $4_{II} + 1_I$ ; and in figure 5e a first metaphase shows  $4_{II} + 1_I$  with one pair loosely joined.

TABLE 1  
SUMMARY OF CHROMOSOME BEHAVIOR IN  $F_1$  HYBRIDS

| F <sub>1</sub> Hybrid                                 | Number of Bivalents at I-M |    |    |    |       |        | Total PMC | Per cent* tetrad irregularities |
|---|----------------------------|----|----|----|-------|--------|-----------|---------------------------------|
|   | 0                          | 1  | 2  | 3  | 4     | 5      |           |                                 |
| <i>C. leontodontoides</i> x <i>tectorum</i>           | 11                         | 10 | 14 | 8  | 11    | .....  | 54        | 7                               |
| <i>C. leontodontoides</i> x <i>parviflora</i> .....   | 6                          | 0  | 5  | 4  | 11    | ... .. | 26        | 7                               |
| <i>C. leontodontoides</i> x <i>capillaris</i> .....   | 11                         | 4  | 5  | 5  | ..... | .....  | 25        | 23.5                            |
| <i>C. leontodontoides</i> x <i>marschalli</i> .. .. . | 17                         | 9  | 13 | 12 | 5     | ... .. | 56        | 20.5                            |
| <i>C. leontodontoides</i> x <i>aurea</i> .....        | 0                          | 0  | 0  | 2  | 12    | 39     | 53        | .7                              |

\*Not including micronuclei.

The pairing is clearly between the chromosomes of the two species and not within the haploid set of either parent. Thus where there is  $1_{II} + 7_I$ , three large *tectorum* and four small *leontodontoides* chromosomes remain unpaired (fig. 4f); where there are  $2_{II} + 5_I$ , two large *tectorum* and three small *leontodontoides* chromosomes remain unpaired (fig. 5a); and with  $3_{II} + 3_I$ , the univalents are one large *tectorum* and two small *leontodontoides* chromosomes (fig. 5b).

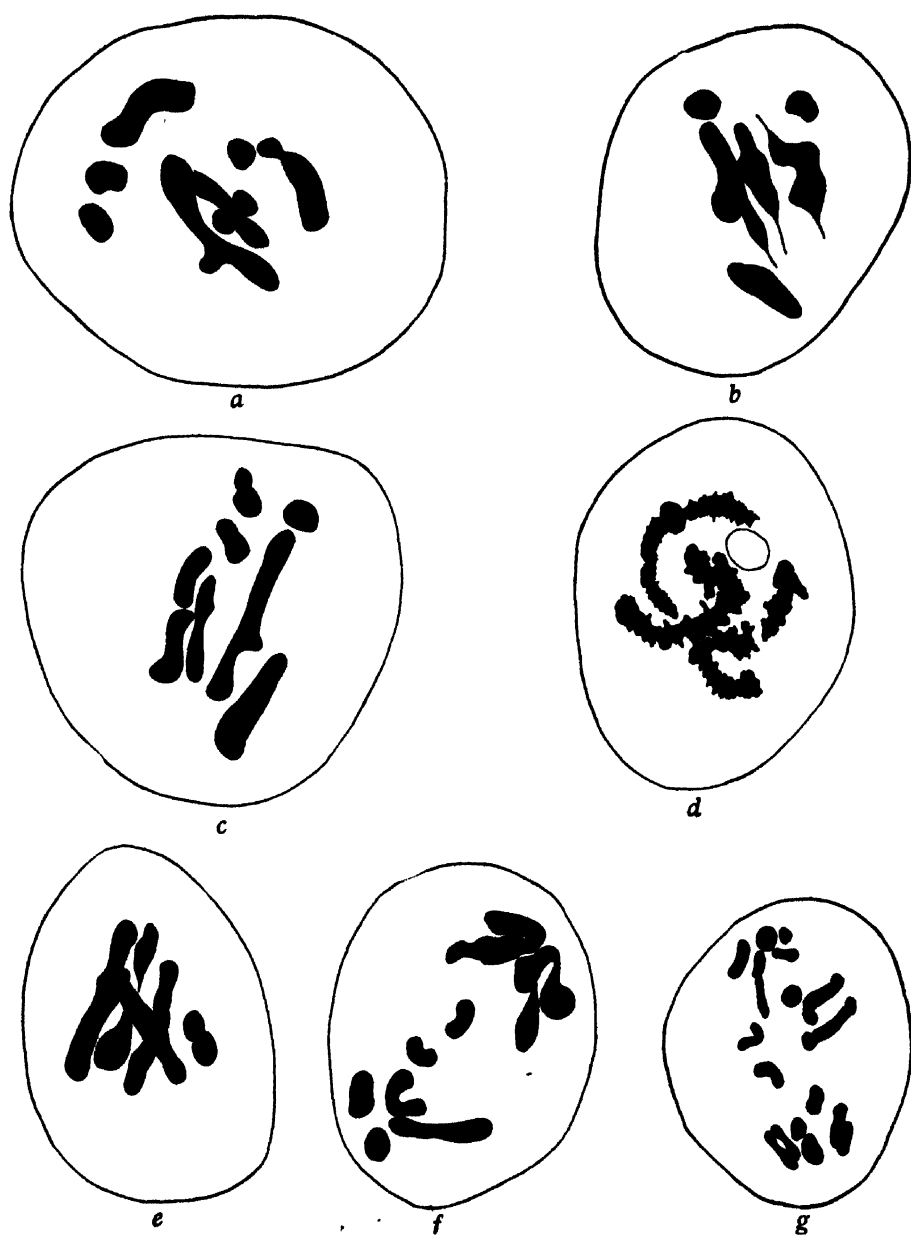


Fig. 5. Meiosis in  $F_1$  *C. leontodontoides-tectorum*.

*a*, I-M, with two bivalents and five univalents; *b*, I-M, with three bivalents, and three univalents; *c*, I-M, with one bivalent, one loose pair, and five univalents; *d*, early diakinesis and *e*, I-M, with four bivalents and one univalent chromosome; *f*, and *g*, late I-A.

The results of such different types of behavior at I-M are evident at I-A where the unpaired chromosomes are frequently seen to lag and divide. Thus a I-A probably following a I-M with  $4_{II} + 1_I$  is shown in figure 5f. Here four chromosomes are at each pole while one small *leontodontoides* chromosome has lagged and divided in the equatorial region. In figure 5g three chromosomes are seen dividing in the equatorial region, while six halves of univalent chromosomes are at each pole. Such an anaphase probably leads to the formation of diploid or near diploid gametes, as evidenced by the presence of 7 per cent dyads at the tetrad stage. Although lagging of unpaired chromosomes is frequent, microcytes are rare at the tetrad stage. Micronuclei are, however, frequent.

The per cent of good pollen grains, filled with protoplasm and taking aceto-carmin stain, varied at different times, from plant to plant and from flower to flower. Under usual conditions, the average per cent of good grains was 5.5 varying from 3.8-10 per cent. After a period of warm weather, however, the proportion in two plants was found to be 21.3 and 23.7 per cent respectively. Even the apparently good pollen grains, however, were incapable of producing viable offspring, no progeny being obtained from the few seeds set after numerous backcrossings of the hybrid with both parents.

$F_1$  *C. leontodontoides*  $\times$  *C. parviflora*.

SOMATIC CHROMOSOMES

In somatic cells of the hybrid, the two longest (A and B) of the four chromosomes of the *parviflora* parent are distinct from all the



Fig. 6. Somatic metaphase of  $F_1$  *C. leontodontoides* — *parviflora*.

*leontodontoides* chromosomes owing to their greater length. The *parviflora* satellited D-chromosome, however, is shorter than any of the *leontodontoides* chromosomes. This is of interest in connection with its suggested origin by fragmentation from the satellited chromosome of *C. capillaris* (cf. Babcock and Navashin 1930). It retains its satel-

lite on a long thread, and is thus easily identified. The D-chromosome of *leontodontoides* loses its satellite, as in the hybrid with *tectorum*. The C-chromosome of *parviflora* is so similar to the B-chromosome of *leontodontoides* in morphology that distinction between the two is not usually possible (fig. 6).

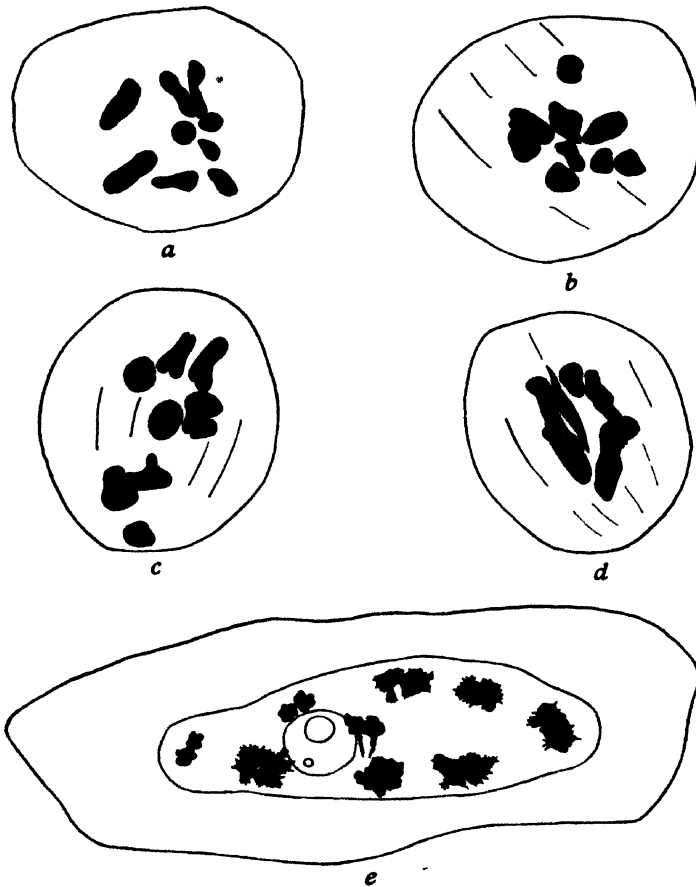


Fig. 7. Meiosis in  $F_1$  *C. leontodontoides-parviflora*.

a, I-M, with no bivalents; b, and c, I-A, showing nine univalent chromosomes; d, I-M, with four bivalents and one univalent chromosome; e, tapetal cell showing diakinesis-like stage.

### MEIOSIS

In the meiotic divisions the distinction between the *leontodontoides* and *parviflora* chromosomes is not so great as in the hybrid with *tectorum*, but the behavior of the chromosomes is rather similar. The same variation in the amount of pairing occurs, but more regular pairing is more frequent. Thus at diakinesis and I-M,  $4_{II} + 1_I$  are seen more frequently than in the hybrid with *tectorum*, but  $9_I$  are also



rather frequent, and intermediate amounts of pairing with  $1_{II} + 7_I$ ,  $2_{II} + 5_I$  and  $3_{II} + 3_I$  occur (figs. 7a-7d). The types of pairing observed in 26 PMC's are listed in table 1.

The average per cent of dyads at the tetrad stage in the plants examined was again 7, but the variation from plant to plant was great and that from bud to bud of the same plant only slightly less. Thus the per cent of dyads varied from .5 to 17.7, the majority of counts showing 3-7 per cent. There were exceedingly few microcytes but micronuclei were frequent. A few triads and pentads were also seen.

The average proportion of good pollen grains in mature pollen was 6 per cent, but the variation was from 1-20 per cent. Flowers on a single plant showed a variation from 4-20 per cent. Most of the good pollen grains were extra large indicating that they came from dyads which probably contained a diploid complement of chromosomes.

In this hybrid a very striking example of a diakinesis-like stage in a tapetal cell was observed. Winge (1917) has found diakinesis-like stages in nuclei of the tapetum of *Humulus japonicus* and other species, which he regards as "a special method of mitotic nuclear division, normally including a typical diakinesis stage, presumably due to anticipated chromosome splitting." The tapetal cell observed in the present case had the size and shape of the normal tapetal cells and was much larger and more oblong than the PMC's. It was observed, however, in an aceto-carmin smear, so that its place of development is unknown. Within the large oval nucleus were nine distinct pairs of short thick chromosomes, lying close to the nuclear membrane (fig. 7e). A vacuolated nucleolus was present as at diakinesis in a PMC. The edges of the chromosomes were somewhat "fringed" as in a typical diakinesis. The fact that nine apparent pairs of chromosomes were present here suggests that a longitudinal division had taken place giving rise to apparent bivalents, the total number of chromosomes being twice that of the somatic cells of the hybrid. This corresponds closely with Winge's observations in *Humulus*, and no doubt here as in *Humulus* the diakinesis-like stage is not followed by reduction, but by further chromosome division.

F<sub>1</sub> *C. capillaris* × *C. leontodontoides*.

## SOMATIC CHROMOSOMES

In somatic cells of the hybrid, the three chromosomes of *capillaris* are morphologically distinct from the *leontodontoides* chromosomes, all three being larger than any of the *leontodontoides* chromosomes. The *capillaris* chromosomes maintain their individuality, the satellite always being present on the D-chromosome. However, the satellite on the D-chromosome of *leontodontoides* was never seen (fig. 8).

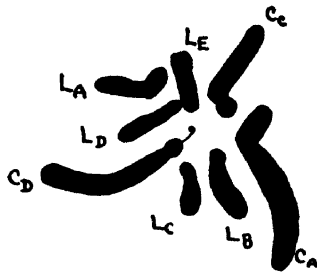


Fig. 8. Somatic metaphase of F<sub>1</sub> *C. capillaris-leontodontoides*.

## MEIOSIS

The great difference in size between the *capillaris* and *leontodontoides* chromosomes makes it possible to distinguish the chromosomes of the two parents in PMC's as well as in somatic tissues. Thus in figure 9a three large *capillaris* and five smaller *leontodontoides* chromosomes are seen in an early metaphase where no pairing occurred. As a rule the meiotic divisions in this hybrid are very irregular. Eight unpaired chromosomes can frequently be seen at diakinesis or early first metaphase, and only very rarely can any true pairing be found, although a loose association at I-M occasionally takes place. Thus in figure 9b two typical pairs have been formed and another pair of chromosomes is loosely associated leaving two small *leontodontoides* chromosomes unpaired.

It is more usual to find some or all of the chromosomes dividing in the first division, and apparently the unpaired chromosomes frequently enter a distributional anaphase in which they may divide without having formed as definite a I-M plate as that shown in figure 9a. Thus in figure 9c, a 4-4 distribution is taking place at I-A but

four of the chromosomes have already divided, and the other four are in the process of division, probably resulting in an interkinesis somewhat like that in figure 9*d*. Here there was probably an 8-8 distribution of halves of univalent chromosomes, but in one nucleus three chromosomes are again dividing and in the other nucleus one chro-

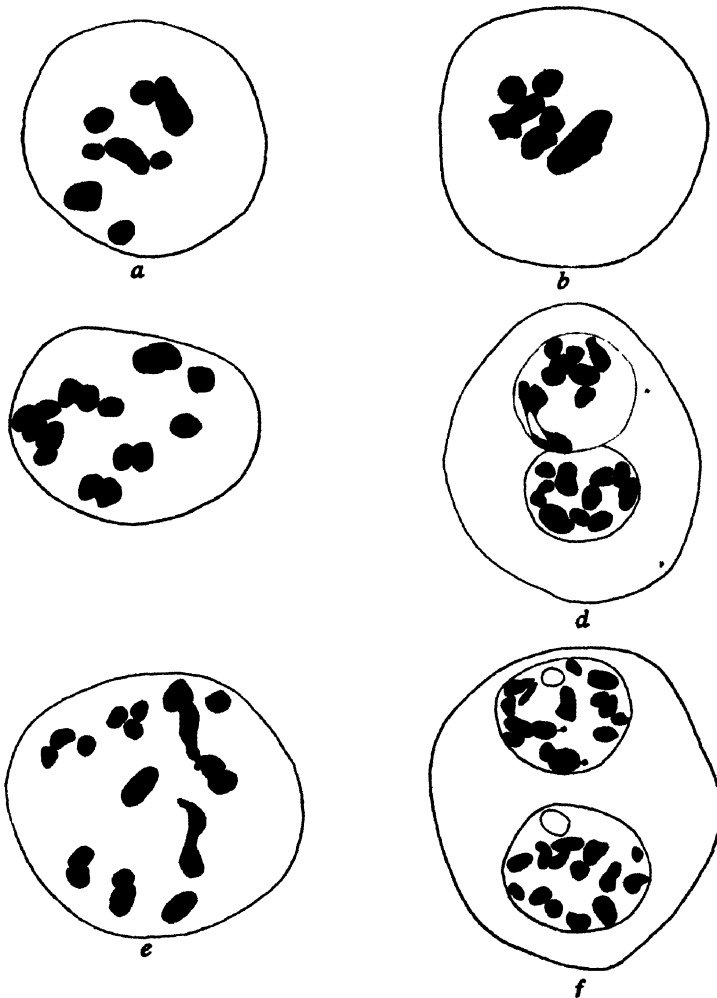


Fig. 9. Meiosis in  $F_1$  *C. capillaris-leontodontoides*.

*a*, I-M, with no pairing between the three large *capillaris* and five small *leontodontoides* chromosomes; *b*, I-M, with two bivalents, one loose pair, and two univalents; *c*, I-A; *d*, interkinesis, showing products of double division or fragmentation; *e*, I-A, where fragmentation has given rise to at least 20 units; *f*, interkinesis, at least fourteen units in each nucleus.

mosome appears to be doubly dividing. In this one large chromosome both longitudinal and cross-division seem to be taking place, and many other PMC's give evidence of fragmentation as well as early

division of chromosomes. Thus figure 9e apparently corresponds to a I-A but division or fragmentation is giving rise to at least twenty units. That double division or fragmentation occurs in the first meiotic division is shown by the interkinesis in figure 9f, where at least fourteen units, some extremely small, are seen in each nucleus.

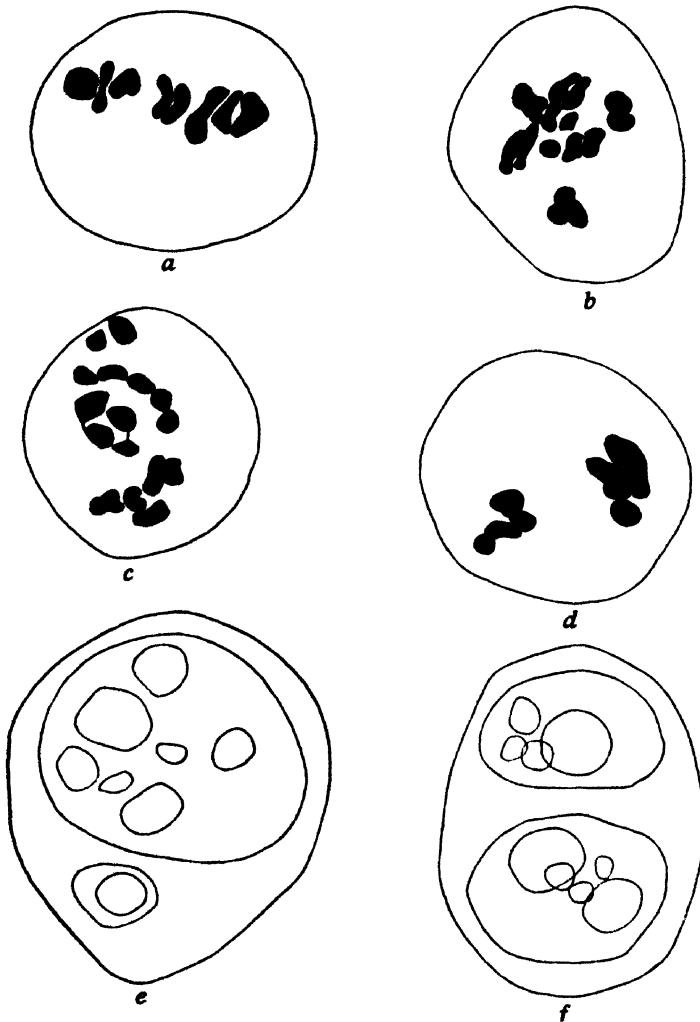


Fig. 10. Meiosis in  $F_1$  *C. capillaris-leontodontoides*.

*a*, I-M, eight univalent chromosomes forming an equatorial plate; *b*, late I-M, eight univalent chromosomes dividing; *c*, I-A, irregular division of chromosomes; *d*, I-T, showing 4-4 distribution of univalent chromosomes; *e*, monad, with microcyte, five large, and three small nuclei; *f*, dyad with three large and six small nuclei.

Occasionally a metaphase is found with the eight chromosomes forming an equatorial plate and undergoing division, as shown in figures 10a and 10b. Usually, however, the division seems to be

accomplished more irregularly at various stages of I as shown in figures 9e, 9f and 10c. The latter figure shows a I-A where six chromosomes have already divided, five halves being at one pole, two at the other, and five in between the plates, and in addition two chromosomes are just completing their division in the equatorial region. Such irregular division leads to various numbers of chromosome units at II-M. Only rarely are I-T or II-T stages seen in which a total of only eight units can be identified, but the PMC in figure 9d shows a I-T with four units in each plate, no division or fragmentation having taken place.



Fig. 11. Somatic metaphase of  $F_1$  *C. leontodontoides-marschalli*.

The irregular meiotic behavior is reflected in the tetrad stage. Micronuclei are frequent in all cells of the tetrads, and where four cells are formed there is considerable variation in their size. Dyads and triads are frequent, an average of about 22.4 per cent being counted, the variation in four buds from one plant being from 17.3 to 26.9 per cent. The dyads contain many nuclei or micro-nuclei, eight or nine usually being present. Thus in figure 10e, a monad with a microcyte has eight nuclei of various sizes and in figure 10f a dyad has three large and six small nuclei. Such behavior at meiosis can, of course, seldom lead to the formation of good pollen grains. Most of the mature pollen consists of small empty grains, and 3 per cent was the highest number of stainable grains observed.

#### $F_1$ *C. leontodontoides* $\times$ *C. marschalli*.

##### THE SOMATIC CHROMOSOMES

In somatic cells of the hybrid, three of the four *marschalli* chromosomes are distinct from all the *leontodontoides* chromosomes because of their greater length. The satellited chromosome of *marschalli*, however, is of about the same length as the *leontodontoides* chromosomes. It retains its satellite and is thus to be distinguished from the *leontodontoides* chromosomes. The *leontodontoides* satellite

again disappears in this hybrid. In figure 11 a somatic plate from a rather old root is shown. Here all the chromosomes are contracted so that the individuality of the *leontodontoides* chromosomes is not evident, but the distinction between the parental sets is obvious.

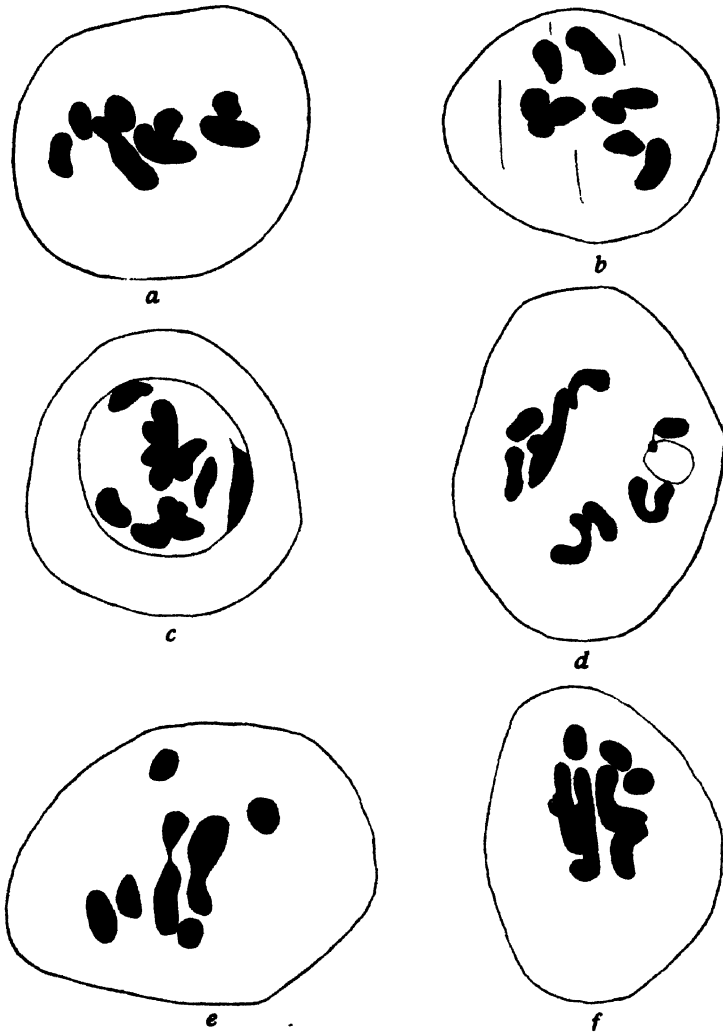


Fig. 12. Meiosis in F<sub>1</sub> *C. leontodontoides-marschalli*.

*a*, I-M, with no bivalents, three of the *marschalli* chromosomes appear larger than the rest; *b*, I-A; *c*, diakinesis with nine unpaired chromosomes; *d*, late diakinesis with one bivalent and seven univalents, one with a satellite on the nucleolus; *e*, I-M, with two bivalents; *f*, I-M, with three bivalents.

#### MEIOSIS

In PMC's of the hybrid the same size relations are found, three large and six smaller units being evident at I-M where no pairing occurs (figs. 12*a* and 12*b*). No pairing of the nine chromosomes is

the most frequent behavior at diakinesis (fig. 12c) and I-M, but here the complete range of types of pairing was observed to take place, there being little significant difference between the frequency of each type of pairing seen. Thus of 56 PMC's in which the type of pairing could be determined, there were 17 with  $9_I$ , 9 with  $1_{II} + 7_I$ , 13 with  $2_{II} + 5_I$ , 12 with  $3_{II} + 3_I$ , and 5 with  $4_{II} + 1_I$ .

In figure 12d, a late diakinesis shows one pair and seven single chromosomes. A small chromosome seems to have a satellite attached which is close to the nucleolus. This is probably the *marschalli* satellited chromosome. Such an origin of satellites on the nucleolus has been described by Kuhn (1928) and Navashin (1927). Figure 12e shows  $2_{II} + 5_I$ . One of the pairs is loosely joined, showing that the synaptic mates are in reality chromosomes quite distinct in size. In figure 12f, there are  $3_{II} + 3_I$ . The three pairs appear to have the characteristic form of bivalents in *Crepis*, although one of the members of each pair is conspicuously smaller than the other. In figure 13a the formation of  $4_{II} + 1_I$  shows that the chromosomes of the two species are occasionally capable of forming normal pairs to the fullest extent possible.

An anaphase in which the three large *marschalli* chromosomes are distinct from the rest is shown in figure 13b. Here no division of univalents has taken place. In figure 13c is shown a II-A after an apparent 6-3 distribution at I-A. Five chromosomes are at each pole in one half of the cell with one delayed chromosome dividing between the plates, and two chromosomes at each pole in the other half of the cell with a chromosome dividing between.

Laggard chromosomes are frequent at I-T and II-T, and result in frequent micronuclei at the tetrad stage. A II-T with five large and two small nuclei is shown in figure 13d. The four cells of the tetrad are often unequal in size. Dyads are frequent and often contain microcytes which vary in size up to that of the other two cells resulting in a triad. The average per cent of dyads and triads was 20.5, ranging from 12.4 to 25.3. The average per cent of good pollen grains was 5.7, and here again most of the good grains were much larger than normal.

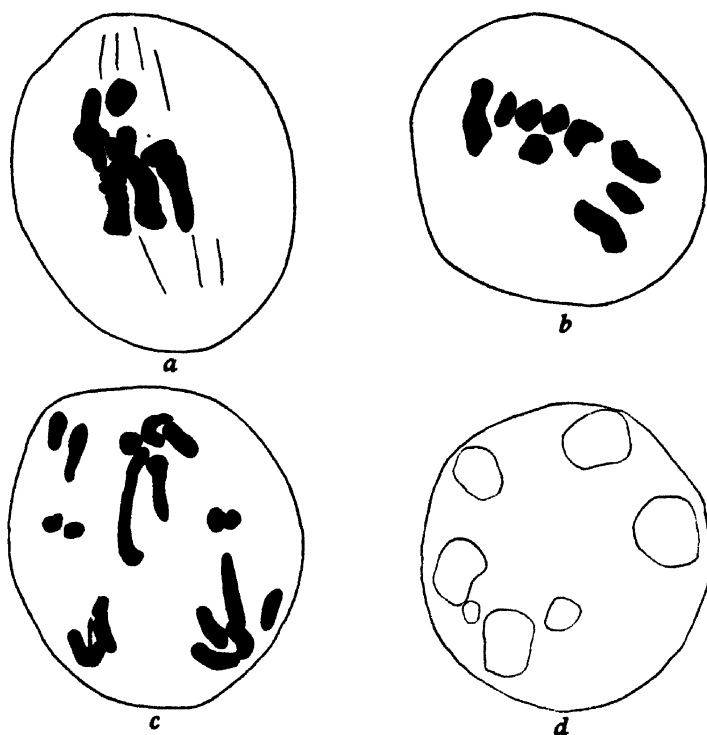


Fig. 13. Meiosis in  $F_1$  *C. leontodontoides-marschalli*.

*a*, I-M, with four bivalents; *b*, I-A, showing three large *marschalli* chromosomes; *c*, II-A, after a 6-3 distribution at I-A; *d*, II-T, five large and two small nuclei.

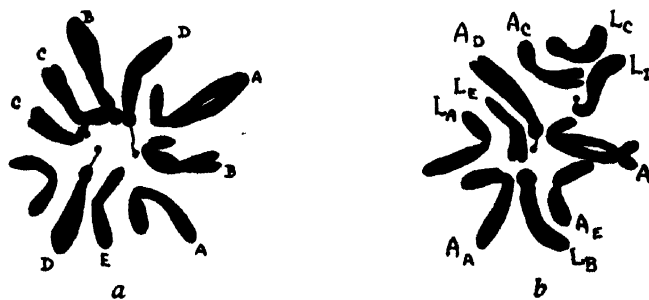


Fig. 14. Somatic metaphases.

*a*, of *C. aurea*; *b*, of  $F_1$  *C. leontodontoides-aurea*.



*F*<sub>1</sub> *C. leontodontoides* and *C. aurea*.

## THE SOMATIC CHROMOSOMES

The chromosomes of *C. aurea* resemble those of *leontodontoides* in more than number, which is  $2n = 10$  in both species. The same morphological classes of chromosomes are found in *aurea* as in *leontodontoides* (fig. 14). Thus the three pairs of long chromosomes are similar in length but are distinguishable on the basis of constrictions and satellites. The A-chromosome has a submedian constriction forming a J-shaped chromosome, while the B-chromosome has a subterminal constriction forming a small head, and the E-chromosome has a median constriction giving it a V-shape. The D-chromosome is nearly equal to the B-chromosome in length, and has a somewhat smaller head to which a satellite is attached, while the C-chromosome is somewhat shorter and has a subterminal constriction deeper than that of the D-chromosome. The whole complex bulks somewhat larger than that of *leontodontoides*, but the types of chromosomes are the same. The size differences within the sets are not the same, however, the most conspicuous difference being in the comparatively greater length of the D-chromosome in *aurea*. The satellite is also larger and usually attached by a longer thread than in *leontodontoides*. Each of the chromosomes of *aurea* is slightly larger than the corresponding chromosome of *leontodontoides*.

In somatic cells of the hybrid there is little difference between the two parental sets of chromosomes, and it is only rarely that every chromosome can be definitely assigned to one or the other of the parental sets. The A- and B-chromosomes of *aurea* usually appear a little larger than the rest of the chromosomes, and the larger satellite makes the D-chromosome of *aurea* distinguishable. The C- and E-chromosomes of the two sets appear so much alike, however, that only rarely is the slight difference in size evident enough to make distinction possible. In most plates the only visible satellite is that of *aurea*, but in several instances the small satellite of *leontodontoides* could also be seen although it was always close to the body of the D-chromosome and never separated from it by the usual thread. It therefore seems that the satellite of *leontodontoides* does not disappear so completely in this hybrid as in the others examined (fig. 14b).

## MEIOSIS

In meiotic divisions the chromosomes of the two species cannot be distinguished. In contrast to the other hybrids there is great regularity in meiosis. At diakinesis  $5_{II}$  or  $4_{II} + 2_I$  are seen. In figure 15a one pair of chromosomes appears to be only loosely associated

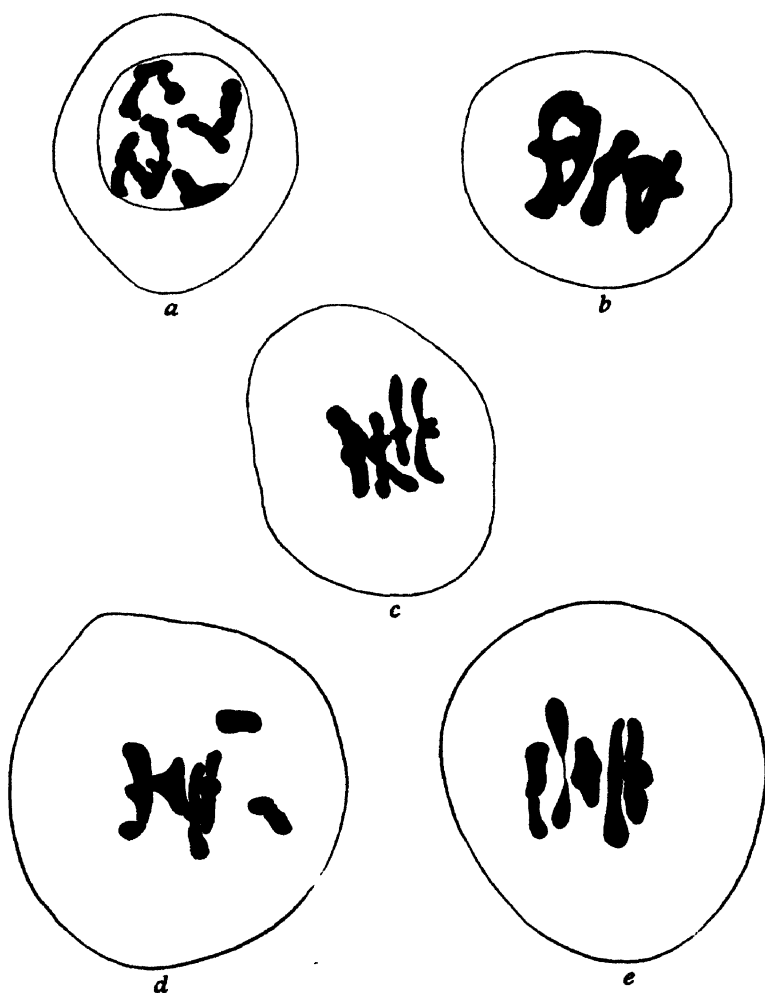


Fig. 15. Meiosis in  $F_1$  *C. leontodontoides-aurea*.

a, diakinesis, with five pairs, one pair loosely associated; b, and c, I-M, with five bivalents; d, I-M, with four bivalents; e, I-M, with four bivalents and one pair loosely conjugated.

while the other four pairs are normally conjugated. The same amount of variation in pairing is found at the first metaphase,  $5_{II}$  being most frequent (figs. 15b and 15c), but  $4_{II} + 2_I$  also occur (fig. 15d) together with many cases of four pairs plus two loosely associated chromo-

soimes (fig. 15e). Rarely two pairs of chromosomes show loose association. Of 53 PMC's in which the type of pairing could be distinguished at diakinesis and I-M, 39 showed  $5_{II}$ , 12 showed  $4_{II} + 2_I$  (including cells showing one loosely joined pair), and two showed three pairs plus two loosely associated pairs.

As would be expected from such behavior at I-M, the I-A and II-A show few irregularities. Occasionally a single chromosome lags, or a pair is late in disjoining at I-A. One or two chromosomes may rarely be left out of the daughter nuclei at II-T. The tetrads are consequently very regular as compared with those of the other hybrids. Micronuclei are occasionally seen, but microcytes are extremely rare. Dyads are formed as in the other hybrids, but to a very small extent, 2 per cent being the highest number counted, and .5 per cent being the average number in 2158 PMC's.

The number of good stainable pollen grains was exceedingly few in all seven hybrids examined. The range observed was from 1.8 to 3.7 per cent, the most showing 2-3 per cent. Some other factors besides irregularities in chromosome distribution must be operating here to produce inviable pollen grains. The per cent of good grains might have been higher under more favorable weather conditions, as was found in the case of other hybrids. At any rate, a larger per cent of female gametes must have been capable of functioning since these hybrids set more viable seed than any others upon open pollination when growing next to *C. leontodontoides*.

The contrast between the *leontodontoides-aurea* hybrid and all other hybrids studied is great. The partial fertility permitting recombinations of parental characters in backcrosses of  $F_1$  is probably directly connected with the more regular meiosis due to the greater amount of normal pairing. This was the only hybrid both of whose parents had somatic chromosomes of the same number and types, and of very nearly the same size. The fact that morphological similarity between the somatic chromosomes is followed by conjugation of the chromosomes to a large extent at meiosis would indicate that here morphological similarity of the chromosomes bears some relation to genetic homology.

## RESULT OF CROSSES OF *C. LEONTODONTOIDES* WITH *C. TINGITANA*

Because of the apparent relationship between *C. leontodontoides* and *C. aurea*, attempts were made during two seasons to obtain hybrids between *C. leontodontoides* and *C. tingitana*, another species with five pairs of chromosomes belonging to the subgenus *Catonia* and morphologically close to *aurea*. No hybrids were obtained the first year, and the second year more extensive attempts were made using various plants of both species. However, no hybrids were obtained from crosses involving sixty-two heads.

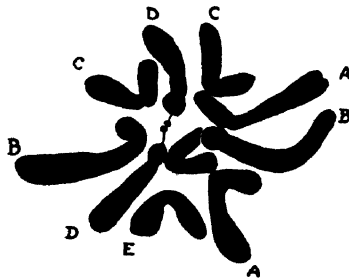


Fig. 16. Somatic metaphase of *C. tingitana*.

The failure to obtain hybrids between *leontodontoides* and *tingitana* does not necessarily mean that the relation between the two species is too distant for the production of such a hybrid. The comparative morphology of their somatic chromosomes would suggest, however, that there is a greater difference between the chromosomes of *C. leontodontoides* and *C. tingitana* than between *C. leontodontoides* and *C. aurea*.

All the chromosomes of *tingitana* are considerably larger than the corresponding chromosomes of *leontodontoides* and *aurea*. The difference in size between the chromosomes of *tingitana* and *aurea* is much greater than that between those of *aurea* and *leontodontoides*. However, the same five types of chromosomes are found in *tingitana* as in the other two species. The satellited chromosome is somewhat shorter here as compared with the A- and B-chromosomes than in the other species, being very nearly of the same length as the D-chromosome of *aurea*. With this exception the size relations within the set

are close to those found in the other two species, but the total length of the chromosomes, and the total bulk of chromatin, are much greater in *tingitana* (fig. 16). In figure 17 one chromosome of each type has been drawn from a single somatic cell of each of the three five paired species. Here the similarities and differences in the chromosome complements are evident.

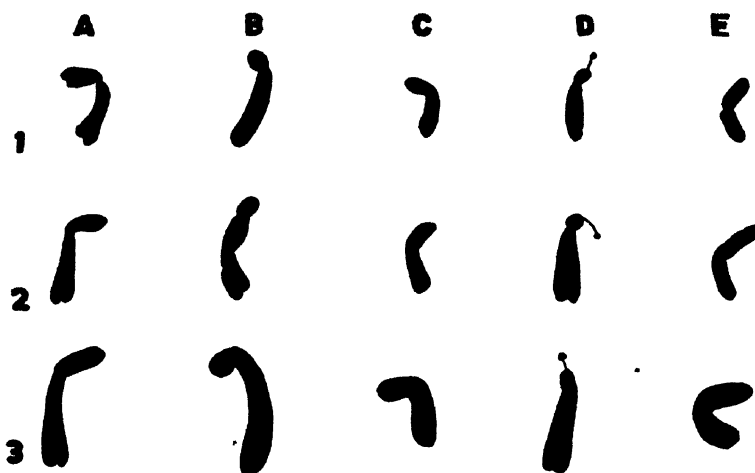


Fig. 17. Somatic chromosomes of (1) *C. leontodontoides*, (2) *C. aurea*, (3) *C. tingitana*. The chromosomes which are similar morphologically are placed under the same letter designations.

## DISCUSSION

Three features observed in these five  $F_1$  hybrids deserve consideration, namely, the sharpness of the distinction in morphology between the chromosomes of the parental sets involved, the conjugation of morphologically distinct chromosomes, and the variable amount of chromosome conjugation. The difference in size between the chromosomes of the paternal and maternal sets is sufficiently great to permit the recognition of practically all the chromosomes as belonging to one genom or the other throughout not only the somatic but also the meiotic divisions in all the hybrids except the  $F_1$  *leontodontoides-aurea*. For this reason the study of the behavior of the parental chromosomes in the meiosis of the hybrids is of particular interest.

The pairing of chromosomes in these hybrids takes place between chromosomes which are morphologically unlike, and is variable in its extent. This raises the question as to the fundamental nature of the synaptic union, but only indirectly contributes to the solution of this increasingly important problem.

The large number of careful observations which have been made on the meiotic phases in plants and animals in recent years, together with evidence from genetic and taxonomic studies, has led to the development of the view that when the chromosomes of the two parental sets can be shown to be composed of essentially similar genic material, and are morphologically alike, they will conjugate to form pairs of chromosomes in the prophase of the meiotic division. It is for this reason that all the chromosomes of a pure species normally conjugate to form pairs. Whether genic dissimilarity will cause chromosomes to fail to pair when it has reached a certain point, is not clear from the experimental and observational evidence. Nevertheless, the view of Gates (1928) seems justified, that the conjugation of two chromosomes is evidence of the mutual specific attractions between their similar genes, while lack of conjugation shows dissimilarity in genic constitution. This familiar point of view will be referred to as the "genic attraction" theory.

In the case of species hybrids, different degrees of conjugation between the parental chromosomes have been observed, and it is necessary to explain the differences according to the genic attraction theory if this theory be accepted for the pairing of chromosomes in a pure species. A few species hybrids are known (e.g. *Nicotiana glauca*  $\times$  *langsdorffii*) in which all the chromosomes of one parent pair with all the chromosomes of the other parent. In these cases the chromosomes of the two species are of the same number and of similar morphology. According to the genic attraction theory, we must infer that the basic genic constitution of the conjugating chromosomes of the two species is similar, and account for divergences in external characters leading to specific distinction through gene mutations distributed throughout the respective genomes. The number of gene mutations in such cases is supposed to be within the limits which affect chromosome conjugation.

In species hybrids where the parental species differ in chromosome number and the *Drosera* type of chromosome conjugation occurs, it would seem that the precise pairing of all the chromosomes of the species of lower chromosome number with an equal number of chromosomes of the species with the high number indicates a similar genic constitution of the conjugating chromosomes. Accordingly, it is possible to suggest that the species with the higher chromosome number has been derived by amphidiploidy from a hybrid between two species with lower chromosome number, one of which or a derivative of

which, was a parent of the hybrid concerned. *Nicotiana tabacum* is a species which may have been derived in this way according to Goodspeed and Clausen (1928), who have used the type of pairing found in the hybrids between *N. tabacum*, *N. sylvestris*, and *N. tomentosa* to support this suggestion.

In rare cases (e.g. *Crepis biennis* × *setosa*) it has been found that the chromosomes of one of the parental species conjugate *inter se* in the F<sub>1</sub> hybrid with another species. Such behavior suggests the polyploid nature of the species whose chromosomes exhibit autosyndesis, for the duplication of a chromosome complement followed by only slight alterations within each complement would allow the chromosomes to attract each other when independent of their more genically similar homologues.

TABLE 2

THE OCCURRENCE OF A VARIABLE NUMBER OF PAIRS OF CHROMOSOMES IN MEIOSIS IN PLANTS

|  | Parental Chromosome Numbers | Number of pairs at I-M | Investigator                   |
|--|-----------------------------|------------------------|--------------------------------|
| <i>Aegilops ovata</i> × <i>Triticum vulgare</i> .                | 14+21                       | 0-3                    | Bleier, 1928.                  |
| <i>ovata</i> × <i>Triticum monococcum</i>                        | 14+ 7                       | 0-5                    | Bleier, 1928.                  |
| <i>ventricosa</i> × <i>Triticum villosum</i> . . . . .           | 14+ 7                       | 0-4                    | Bleier, 1928.                  |
| <i>Crepis aspera</i> × <i>bursifolia</i> . . . . .               | 4+4                         | 0-4                    | Babcock and Clausen, 1929      |
| <i>aspera</i> × <i>aculeata</i> . . . . .                        | 4+4                         | 3-4                    | Babcock and Clausen, 1929      |
| <i>taraxaeifolia</i> × <i>tectorum</i> . . . . .                 | 4+4                         | 1-4                    | Babcock and Clausen, 1929      |
| <i>capillaris</i> × <i>tectorum</i> . . . . .                    | 3+4                         | 0-3                    | Hollingshead, 1930a            |
| <i>Nicotiana rustica</i> × <i>tabacum</i> . . . . .              | 24+24                       | few                    | Christoff, 1928                |
| <i>bigelovii</i> × <i>nudicaulis</i> . . . . .                   | 24+24                       | 4-14                   | Webber, 1927                   |
| <i>Papaver atlanticum</i> × <i>dubium</i> . . . . .              | 7+14                        | 1-3                    | Ljungdahl, 1922                |
| <i>somniferum</i> × <i>nudicaule</i> . . . . .                   | 11+7                        | 3-4                    | Yasui, 1927                    |
| <i>Polypodium aureum</i> × <i>vulgare</i> . . . . .              | 34+90                       | 0-30                   | Farmer and Digby, 1910         |
| <i>Solanum nigrum</i> haploid . . . . .                          | 36                          | 3-12                   | Jørgensen, 1928                |
| <i>Triticum dicoccum</i> × <i>monococcum</i> . . . . .           | 14+7                        | 4-7                    | Kihara, 1924                   |
| <i>dicoccum</i> × <i>aegilopoides</i> . . . . .                  | 14+7                        | 4-7                    | Kihara, 1924                   |
| <i>turgidum</i> × <i>monococcum</i> . . . . .                    | 14+7                        | 3-7                    | Thompson, 1926                 |
| <i>spelta</i> × <i>monococcum</i> . . . . .                      | 21+7                        | 0-5                    | Melburn & Thompson, 1927       |
| <i>vulgare</i> × <i>Secale cereale</i> . . . . .                 | 21+7                        | 0-3                    | Kihara, 1924<br>Thompson, 1926 |
| <i>Verbascum blattaria</i> × <i>Celsia bugulifolia</i> . . . . . | 15+17                       | 12-15                  | Håkansson, 1926                |
| <i>Viola arvensis</i> × <i>tricolor</i> . . . . .                | 17+13                       | 13-15                  | Clausen, 1922                  |

The table given by Renner (1929, p. 107) was used as a basis for the list given here.

Complete failure of all the chromosomes of one set to pair with any of the chromosomes of the other set is not of infrequent occurrence in interspecific hybrids. This occurs where the chromosomes of the parents are the same in number and morphology, or different. In the first case, the presumption is that the species have become distinct through the accumulation of genic differences of sufficient magnitude to destroy the mutual attraction of homologous chromosomes. Thus in the case of the hybrids between *Raphanus* and *Brassica* (Karpechenko 1927) where the parental species belong to different genera, the chromosomes of the two genomes are similar in number and morphology but fail to show any mutual attraction at the heterotypic division. Where the chromosomes of the two genomes which fail to pair differ in number or morphology or both, other processes besides gene mutation have obviously been concerned in the differentiation of the chromosome complements of the species. These processes may be included under the general term "transformations," and will be considered below.

The formation of a variable number of pairs between the chromosomes of the two parental sets, whether they are of the same or of different number and morphology, has been reported as occurring in a number of interspecific hybrids. A list of instances of variable pairing in the case of plant hybrids is given in table 2. The significance of this type of behavior has been variously interpreted (cf. Farmer and Digby, 1910; Harrison and Doncaster, 1914; and Winge, 1917), but no interpretation in accordance with modern genetic and cytological knowledge has as yet been suggested which seems adequate to account for the occurrence of the variable pairing characteristic of the *Crepis* hybrids as described above.

The evolutionary processes which it seems reasonable to assume have been largely responsible for the differentiation of the distinct chromosome complements of *Crepis* species, must also be responsible for the differences between the genomes of the species which cause the formation of a variable number of pairs of chromosomes in meiosis in their hybrids. Hollingshead and Babcock (1930), after a study of the somatic complements of some seventy *Crepis* species, have pointed out that the differences between the genomes of these species can be accounted for only on the basis that transformational processes have been at work which induce changes in chromosome number and morphology. These transformational processes include mechanical changes in chromosome structure, such as inversion, trans-



location, deletion, duplication, union, and fragmentation, which have been observed to occur both under natural conditions and after subjection to high frequency radiations in *Drosophila* and *Datura*.

An illustration of the extent of the change in a chromosome complement which these processes might produce if operating over long periods of time is given in figure 18. Here, through the interference of fragmentation, translocation, inversion, deletion, and duplication, a genom has been evolved which differs in chromosome number, size, and shape, from the original complement. Yet the chromosomes of this "transformed" genom possess segments which are genically similar to segments of the chromosomes of the original complement.

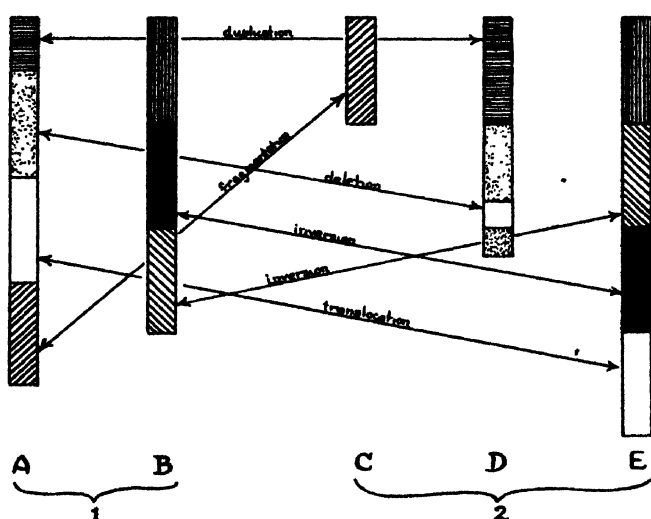


Fig. 18. Diagram showing the possible change in chromosome number and size of a genom brought about through transformational processes. Chromosome segments which are similar in genic constitution in the original and the transformed genom are similarly shaded.

These segments are now borne by chromosomes of different length and morphology. These processes operating under different external conditions over a similar period of time on the same original chromosome complement would produce transformed genomes of other types, all of which, in common with the two illustrated, would possess segments of chromosomes essentially alike in genic constitution. The similar genes borne by different transformed genomes must be responsible for the generic characters common to the species possessing these different genomes. Deletions and duplications of genes, together with gene mutations taking place concurrently with these transformational processes, and not illustrated in the diagram, may in most cases be responsible for the character differences between the species.

According to this view, pairing between the chromosomes of complements which are not alike as to number or morphology is due to the residual genic similarity of these chromosome segments. The larger or more numerous the segments which are genically similar in the case of two chromosomes belonging to different species, the greater is their mutual attraction and the more constant their pairing, while the smaller or fewer the similar segments, the weaker is their attraction and the more variable their pairing, ending in complete failure to pair when the genic similarity is less than a certain minimum. Thus we might explain different extents of chromosome conjugation in the meiotic divisions of the hybrid between two species with the chromosome complements shown in figure 18, upon the assumption that the chromosomes of the last archesporial division enter the resting stage with their orientation with reference to one another a chance one. The segments of chromosome A of species 1 genically similar to those of chromosome D of species 2 might then be of sufficient extent to attract this chromosome and permit at least loose conjugation with it, if the two chromosomes are near each other in the early prophase of the heterotypic division. If, however, the two chromosomes are widely separated, the mutual attractions of their genes may be insufficient to permit the chromosomes to conjugate.

The question as to whether or not the chromosomes in mitosis and meiosis are arranged in the spireme stages and the metaphase plates purely by chance, or assume a more definite arrangement dependent upon homology or size, does not seem to have been adequately answered as yet. Kuwada (1929) and his co-workers have shown that the chromosomes in meiosis tend to assume an arrangement characteristic of a like number of magnetized needles of corresponding sizes in a polarized field. Accordingly, the arrangement of chromosomes in both mitosis and meiosis is supposed to be rather definite, and determined by chromosome size and number. Although definite space relations may be the rule, it would seem that in hybrids such as those of *Crepis* where the chromosomes form a graduated series as to size, there must be several alternative balanced arrangements. The orientation of any one of the large chromosomes in reference to the other chromosomes must also be dependent somewhat on chance, so that proximity in prophase might well be the deciding factor in the conjugation of two chromosomes containing genically similar segments. Cleland (1928) has shown that in *Oenothera* it is probable that the chromosomes are definitely arranged in the early prophase

of the meiotic division according to their homologies and parental derivation, but no cytological proof of such definite arrangement in early prophase has been secured in *Oenothera* or any other organism.

Thus the evidence from the mode of chromosome conjugation in meiosis of interspecific hybrids supports the conclusion drawn from the study of the somatic chromosome complements of sixty-seven species that chromosome transformation has been an important method of differentiation of specific genomes in *Crepis*.

### SUMMARY

1. Hybrids between *Crepis leontodontoides* and species belonging to three subgenera were studied cytologically in both somatic and meiotic phases. *C. leontodontoides* ( $n=5$ ) belongs to the subgenus *Eucrepis* and the  $F_1$  hybrids studied were made with the *Eucrepis* species *tectorum* ( $n=4$ ), *parviflora* ( $n=4$ ) and *capillaris* ( $n=3$ ); with the *Barkhausia* species *marschalli* ( $n=4$ ), and the *Catonia* species *aurea* ( $n=5$ ).

2. The chromosomes of the two parental species can be distinguished in somatic figures in all the hybrids, although the distinction is slight in the  $F_1$  *C. leontodontoides-aurea*.

3. The somatic chromosomes of *C. leontodontoides* and *aurea* are of the same morphological types, but those of *aurea* are slightly larger. The chromosomes of *C. tingitana*, a *Catonia* species close to *aurea*, are of the same number and shape as those of *leontodontoides* and *aurea* but are considerably larger. Efforts to secure hybrids between *C. leontodontoides* and *tingitana* were unsuccessful.

4. The satellite disappears from the D-chromosome of *leontodontoides* in the  $F_1$  hybrid with *tectorum*, *parviflora*, *capillaris*, and *marschalli*, but is sometimes evident close to the D-chromosome in the  $F_1$  *leontodontoides-aurea*.

5. All the chromosomes can be identified as belonging to one or the other of the parental genomes throughout meiotic as well as somatic divisions, in the  $F_1$  *capillaris-leontodontoides*, and the  $F_1$  *leontodontoides-tectorum* owing to the great size difference between the chromosomes of the two complements. In the  $F_1$  *leontodontoides-parviflora* and the  $F_1$  *leontodontoides-marschalli* all but one or two of the chromosomes of the species with larger and fewer chromosomes can be identified in the meiotic divisions.

6. The number of bivalents formed in meiosis varied in different PMC's from complete conjugation between the parental chromosomes to entire absence of conjugation in all hybrids except the  $F_1$  *leontodontoides-aurea*. In this hybrid, the greater extent and regularity of chromosome conjugation indicates that the morphological similarity of the chromosomes bears some relation to genetic homology. In all other hybrids the pairing is obviously between chromosomes of dissimilar morphology.

7. It is suggested that the variable pairing characteristic of these hybrids is a reflection of the transformational processes presumably responsible for the differentiation of the specific genomes.

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**THE INTERSPECIFIC HYBRID, CREPIS  
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**BY**

**CHARLES F. POOLE**

**UNIVERSITY OF CALIFORNIA PUBLICATIONS IN AGRICULTURAL SCIENCES**  
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# THE INTERSPECIFIC HYBRID, *CREPIS* *RUBRA* × *C. FOETIDA*, AND SOME OF ITS DERIVATIVES. I

BY

CHARLES F. POOLE

## INTRODUCTION

The investigation dealt with in the following paper concerns a cross between two species in the subgenus *Barkhausia* of the genus *Crepis*, both species having five pairs of chromosomes which exhibit, in somatic cells, size and shape differences in two of their five pairs. In addition, the members of the haploid sets show decided differences between one another.

*C. foetida* has four pairs similar in shape to four from *C. rubra*, but it lacks one corresponding to the fifth *rubra* chromosome, its place being taken by a duplication of another. Furthermore, one of the *rubra* satellited chromosomes frequently lacks its satellite in some strains, a situation existing in the two *rubra* parents represented in this study.

Numerous outstanding differences exist in the external morphology of the two species.

Little previous work has been done on this cross, beyond the determination of chromosome individuality, the occasional appearance of five bivalent chromosomes in the diaphase of  $F_1$ , and the supposed adherence of this hybrid to Navashin's scheme of "Amphiplastie" which has since been found to be erroneous in this case.

Navashin (1925) studied homology in ten *Crepis* species: one having three bivalents, six having four bivalents, and three having five bivalents. Therein he demonstrated the existence within his four-paired species of a B-chromosome not present in the three-paired species, *C. capillaris*, and also the existence in the five-paired species of an E-chromosome not represented in any species having less than five pairs. Both the species dealt with in the present paper were included in Navashin's study.

*C. rubra* was designated as having a haploid set A, B, C, D, and E, with C and D both satellited, the satellite attached to D being double and decidedly larger than the one attached to C. The smaller satellite itself, in all species except *C. rubra*, is a large well rounded body.

Difficulties in homologizing the chromosomes of these ten species begin when it is considered that the *rubra* chromosome C more nearly approaches in size and shape the D satellited chromosomes of *capillaris*

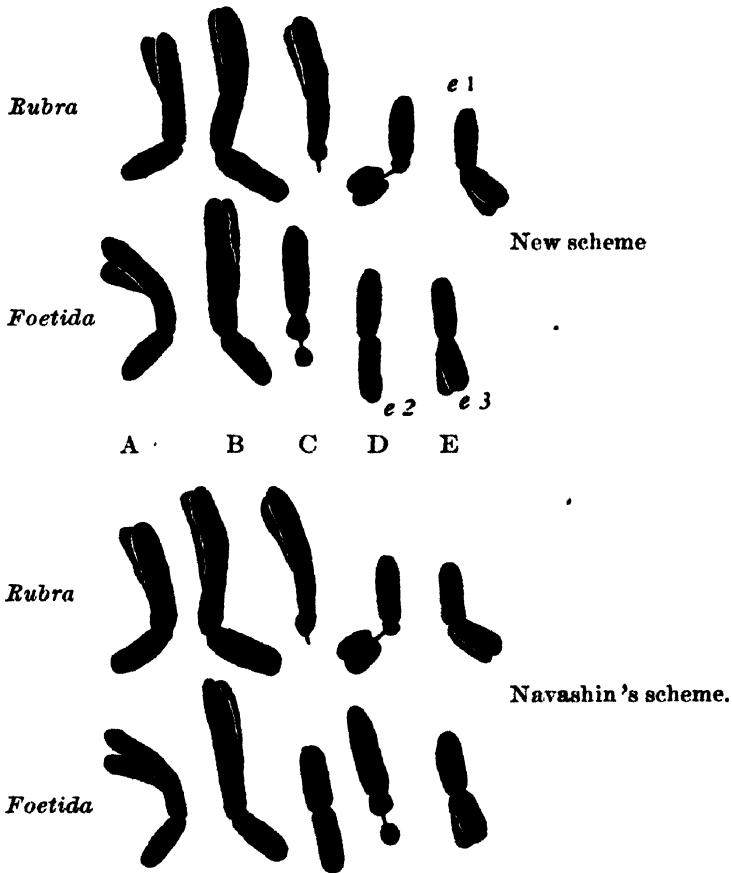


Fig. 1. Proposed schemes of chromosome homology in *C. rubra* and *C. foetida*.

and of all the four-paired species except *parviflora*, as well as the *foetida* chromosome he called D. Furthermore, *rubra* D in his scheme has no counterpart in any of the ten, when we consider the extra large and generally observable double nature of its satellite.

Other difficulties arose in describing the garniture of *C. foetida* subsp. *rhoedifolia*. All the subspecific forms of *Crepis foetida* have the same chromosome garniture. Here the E-chromosome is represented twice in the haploid set, whereas there is no second satellited

chromosome corresponding with *rubra* C. Consequently, Navashin designated the second E as *foetida* C, notwithstanding its obvious lack of relationship to *rubra* C.

The outstanding difficulty, then, lies in reconciling the fact that the F<sub>1</sub> hybrid frequently exhibits complete pairing of five homologues with the fact that the sets will not completely harmonize in Navashin's scheme. A picture of the situation may be obtained from figure 1.

The simplest solution seems to be to accept his designation of *rubra* as: A, B, C, D, and E; give the obviously homologous members of *foetida* the same letter, considering the *foetida* satellited one as the homologue of *rubra* C (which always has a satellite thread and sometimes a small satellite); and consider the extra *foetida* E<sup>2</sup> as homologous with *rubra* D, while the other is designated *foetida* E<sup>3</sup>. As will be seen in the illustrated somatic garnitures, a comparison of *rubra* D with the E-chromosome shows sufficient resemblance to regard it as being a member of the heteromorphic pair which occasionally conjugates at the diaphase of F<sub>1</sub>. The E-chromosomes have a median constriction, usually presenting the appearance of a perfect V. *Rubra* D has a short body ending in a subterminal constriction upon which there is a comparatively heavy thread, holding a double satellite. The total length of this chromosome usually agrees with that of an E-chromosome. This is especially true if we compare the length of the body of *rubra* D with that of one-half of an E-chromosome.

Evidence will be presented to show that the two now being called C both contain factors conditioning color of anther tubes and position of buds before anthesis.

In the following paper certain features of this hybrid and its derivatives will be discussed: (a) types obtained in the somatic garniture, (b) meiotic behavior, (c) the degrees of fertility and sterility, with probable causal agents, (d) morphology of the hybrids, and (e) deductions to be drawn from a combined cytological and genetic study.

## COMPARISON OF THE PARENT SPECIES

The original cross giving rise to the majority of the hybrid derivatives under study, *C. rubra* (strain 1110) × *C. foetida rhoeadifolia* (strain 1539), was made in 1924 by C. W. Haney. Figure 2 shows the somatic garnitures of the two species. This cross will hereafter be referred to as Cross I. F<sub>1</sub> was backcrossed to both parents, with ease to *rubra*, but with difficulty to *foetida* in either direction. Conse-

quently a study of the selfed backcross derivatives must be largely confined to the *rubra* side.

No cytological study was made or notes taken upon the constitution of the  $F_1$ , the backcross generation, or the  $F_2$  from this cross. Study was confined to the selfed backcross generation, all that was available when the present study was commenced. The author has thus far been able to obtain but one plant of the *rhoeadifolia* parent for root tip

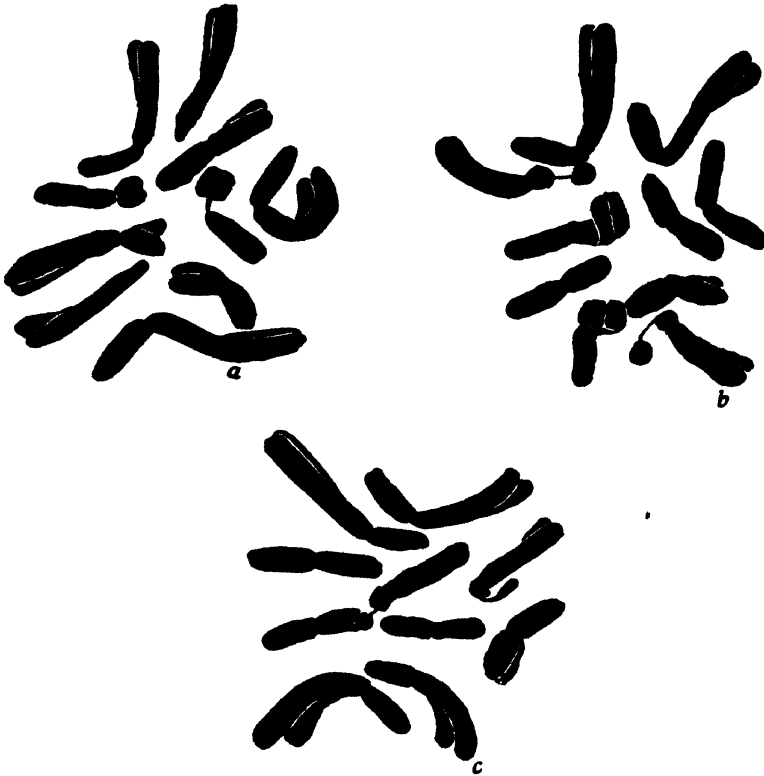


Fig. 2. Somatic garnitures of a, *C. rubra*; b, *C. foetida typica*; c, *C. foetida rhoeadifolia*.  $\times 3300$ .

cytological examination, and this died before reaching maturity. Buds from other plants of this parent strain, 1539, had been fixed previously, however, thus permitting an aceto-carmine study of meiosis in which irregularities were noted, as may be seen from figure 3.

The cytological studies following were made from material fixed and stained as shown below:

A. Root tips: fixed in Navashin 24 hours, stained in Haidenhein's haemotoxylin with standard schedule reported for all the *Crepis* investigations at Berkeley (Hollingshead and Babcock, 1930).

B. Buds: (a) Non-permanent: fixed in Carnoy one hour, and stained with aceto-carmine.

(b) Permanent: fixed in Carnoy 5 minutes; Navashin about 24 hours; stained in haematoxylin, with schedule identical for that of root tips.

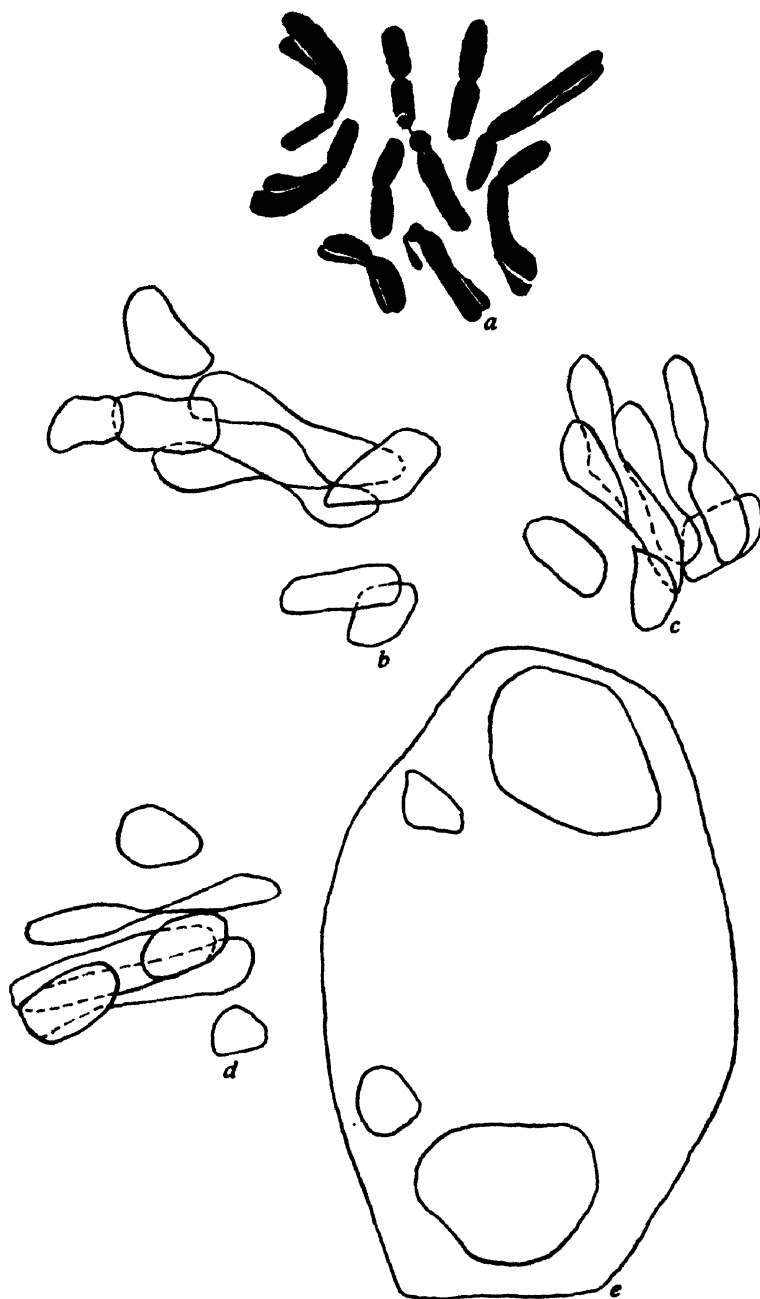


Fig. 3. Somatic and meiotic plates of *C. foetida rhoeadifolia*, strain 1539, used as parent in Cross I. a, somatic garniture; b, IM regular; c, d, showing non-conjunction of one pair; e, interphase, showing elimination of one pair.

From backcross studies it was hoped to identify the *foetida* characters accompanying such *foetida* chromosomes as could be distinguished. At first it was believed there were sufficient cytological differences between the two species to afford critical evidence on more than two pairs of chromosomes, because the members of the *rubra* set are consistently larger than the *foetida* homologues. It soon became evident, however, that no reliability could be placed on constancy of size differences in the hybrids, since three of the homologues, A, B, and  $E^1/E^3$  afforded insufficient heteromorphism for utility in this connection; and furthermore, that, despite numerous external morphological differences, they are mainly of a quantitative nature and relatively few are suitable for correlation studies.

*C. foetida rhoeadifolia*, strain 1539, despite its greater sterility, was chosen as parent in preference to *C. foetida typica* because it offered several character pair contrasts with *rubra* not available in the latter, notably: (a) erect buds, (b) a set of long coarse eglandulose hairs on the involucre bracts, (c) a different shape of the outer bract itself, and (d) a taller plant, frequently exceeding 100 cm., whereas *typica* is not much taller than *rubra*. Except for certain achene differences, a wider distribution of anthocyanin over the stem, branches, and leaves, the remaining characters are those of *foetida typica*.

The *rubra* parent was of the varietal form *alba*, in which the ligules are white, the anther tubes purple or lavender, and the pollen grains white.

In addition to Cross I, a second cross has been utilized, in which *foetida typica* was crossed with a white-flowered strain of *rubra* practically identical with the one occurring in Cross I.

Plate 9, figure a, shows four plants, from left to right: *foetida typica*, F<sub>1</sub> (*foetida typica* × *rubra*), *rubra*, and a derivative of the *foetida rhoeadifolia* parent. The branching habit of all four types is well illustrated and *rhoeadifolia* is seen to be quite luxuriant when mature.

Cross II was performed by M. Navashin in Moscow, and the F<sub>1</sub> was raised in Berkeley. Only one plant was selfed, giving rise to the most interesting of the derivatives thus far obtained.

*Foetida typica* is highly fertile, but *rhoeadifolia* is much less so. A glance at figure 3, a, b, c, and d indicates that the meiotic irregularities occurring in plants of the strain of *rhoeadifolia* used for parent in Cross I may be partly associated with a difference in the



size of the satellites found in every somatic plate, examined from the only plant of strain 1539 available. This, together with some lack of genetic homology, may account for the lower fertility of *rhoeadifolia* compared with *foetida typica*. A single pair of chromosomes shows non-conjunction in meiosis and the somatic garniture indicates that the satellites on the two C-chromosomes are never the same size. Figure 3e is drawn to the same scale as the other meiotic figures and it is evident that the excluded chromatin material adjoining the two interphase nuclei exactly correspond in size to the non-conjugating chromosomes of 3d. Therefore, irregularities in one pair may be causing all the sterility. Pollen grain counts are not yet available in this strain.

### CROSS I

In summarized tabular form, the character contrasts afforded by Cross I (*C. rubra*, 1110 × *C. foetida rhoeadifolia*, 1539) are:

|               | RUBRA  | FOETIDA  |
|---------------|--|--|
| Stems         |  |  |
|               | Stems numerous, almost glabrous, scapose       | Stems solitary, branched, leafy whole length, pubescent        |
|               | No anthocyanin (rarely any)                    | Anthocyanin widespread   |
| Leaves        |  |  |
|               | Rosette dark green, erect, almost glabrous     | Rosette gray green, flat, pubescent                            |
| Buds          |  |  |
|               | Nodding before anthesis                        | Erect before anthesis  |
|               | Outer bracts glabrous                          | Outer bracts with coarse eglandulose hairs                     |
|               | Outer bracts ovate lanceolate, margin scarious | Outer bracts broadly lanceolate, margin not scarious           |
| Flowers       |  |  |
|               | Ligules white, both sides                      | Ligules stroutian yellow, with median red stripe on outer side |
|               | Anther tubes purple                            | Anther tubes yellow  |
|               | Style branches white                           | Style branches greenish brown or yellow                        |
|               | Pollen grains white                            | Pollen grains yellow   |
|               | Open all day                                   | Open forenoon only   |
|               | Achenes purplish brown, inner ones 16-20 mm.   | Achenes light brown, inner ones 12-16 mm.                      |
|               | Diameter open head 47 mm.                      | Diameter open head 29-38 mm.                                   |
| Miscellaneous |  |  |
|               | Height ca. 26 cm.                              | Height ca. 100 cm.   |
|               | Odor slightly <i>foetida</i>                   | Strongly <i>foetida</i>  |

The present study was begun with achenes of (a) the backcross to *rubra* selfed, in which thirty plants reached maturity, from over one hundred germinated (one, a triploid, and the root tips of two others were lost); and (b) the backcross of *foetida* selfed, of which only seven plants germinated or reached maturity. The thirty plants in (a) were derived from six plants of the backcross generation (see table 1). An attempt is being made to duplicate as many as possible of the earlier stages of the investigation.

In the seven plants from (b), the morphology was predominately *foetida*, and the somatic metaphase plates were apparently all of *foetida*-like appearance, with two exceptions to be discussed later. However, so small a generation provides such inadequate means for correlation of cytological and morphological evidence as to be practically useless.

### THE BACKCROSS TO *RUBRA*

With the twenty-eight plants from the backcross to *rubra*, however, the situation is different. Sufficient material is provided to permit rather definite conclusions regarding: (a) distribution of chromosomes from the two original parents, as well as (b) a correlation between such *foetida* chromosomes and characters as are distinguishable.

As previously noted, but two of the five homologues are sufficiently heteromorphic for service in this study, the pairs  $C_rC_r$  and  $DE^2$ . For each pair, then, the backcross population would show a distribution of  $.5 RR + .5 RF$ , which on selfing would give a population distributed in the proportions,  $.625 RR + .25 RF + .125 FF$ . In order to determine the distribution for any given number of pairs of distinguishable chromosomes, this expression may be raised to the corresponding power. In this instance the formula would be represented as  $(.625 DD + .25 DE^2 + .125 E^2E^2) (.625 C_rC_r + .25 C_rC_r + .125 C_rC_r)$ .

Multiplying the two members of this formula will give us types which are readily distinguished in somatic metaphase garnitures, illustrations of which for the two parents may be seen in figure 2, and for  $F_1$  in figure 4. The types existing among the hybrid derivatives may be roughly divided into three main groups corresponding to the two parents and  $F_1$ . The distinction between such types depends solely on the distribution of the pair  $D/E^2$ , and for convenience in subsequent reference such types may be designated by the suffix oid; e.g., (a) rubroid, (b) foetoid, and (c) funoid. Each of these three

groups may in turn be subdivided into three groups according to the distribution of the pair  $C_r/C_t$ . Among these nine types those in which the homologues are heteromorphic may be further distinguished as pseudo-rubroid  $rf$ , or  $ff$ , pseudo-foetoid  $rr$ , or  $rf$ , and pseudo-funoid  $rr$ , or  $ff$ .

Upon this basis comparisons may be made between the number of such somatic types actually observed and the numbers calculated by solution of the formula now under consideration.

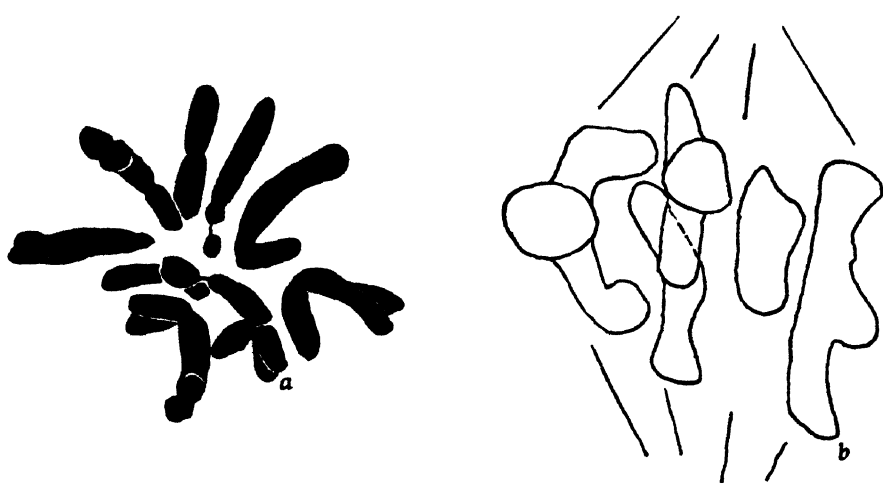


Fig. 4. Somatic and IM plates of an  $F_1$  hybrid between *C. rubra* and *C. foetida typica*. *a*, somatic garniture; *b*, IM, showing five bivalents (aceto-carmin preparation).

TABLE 1  
COMPARISON OF OBSERVED WITH EXPECTED SOMATIC GARNITURE TYPES

| Somatic type  | Observed | Per cent | Calculated |
|---|----------|----------|------------|
| Rubroid $C_rC_rDD$ . . . . .                          | 11       | 39.3     | 39.1       |
| Pseudo-rubroid $C_rC_tDD$ . . . . .                   | 5        | 17.85    | 15.6       |
| Pseudo-rubroid $C_tC_t$ . . . . .                     | 5        | 17.85    | 7.8        |
| Funoid $C_rC_tDE^2$ ( $C_rC_r$ , $C_tC_t$ ) . . . . . | 3        | 10.714   | 25.0       |
| Foetoid $C_tC_tE^2E^2$ . . . . .                      | 2        | 7.143    | 1.6        |
| Pseudo-foetoid $C_rC_tE^2E^2$ ( $C_rC_r$ ) . . . . .  | 2        | 7.143    | 10.9       |

The discrepancies thus obtained are probably due to several causes, chiefly that (a) of the six cultures representing this selfed backcross generation, three have but one plant each and two are fairly well represented, and (b) the six parent plants probably do not represent a random sample of the backcross generation (see table 2).

TABLE 2

CORRELATION OF FOETIDA CHARACTERS WITH FOETIDA CHROMOSOMES IN CROSS I

| Plant No. | Foetida characters   | Somatic type  | Per cent good pollen |
|-----------|--|---|----------------------|
| 28.001-1  | Red stripe, purple tips  | p. rubroid $C_rC_r$   | 82.9                 |
| 3         | Red stripe, anthocyan  | rubroid $C_rC_r$  |                      |
| 4         | Yellow anther tubes, erect buds, anthocyan                                   | p. rubroid $C_rC_r$   |                      |
| 9         | Yellow anther tubes, erect buds  | p. rubroid $C_rC_r$   | 70.5                 |
| 10        | Yellow anther tubes, erect buds  | p. rubroid $C_rC_r$   |                      |
| 11        | Red stripe, anthocyan, long life   | rubroid $C_rC_r$  |                      |
| 12        | Central stem, red stripe, yellow stigmas, anthocyan, hairy leaves, long life | rubroid $C_rC_r$<br>(4n sector)   | 70.5                 |
| 13        | Central stem, red stripe, anthocyan, purple tips                             | p. rubroid $C_rC_r$   |                      |
| 14        | Erect buds, red stripe, yellow anther tubes, long life                       | p. rubroid $C_rC_r$   |                      |
| 16        | Anthocyan, hairy leaves  | rubroid $C_rC_r$  | 70.5                 |
| 18        | Erect buds, yellow tube, red stripe, long life                               | p. rubroid $C_rC_r$   |                      |
| 19        | Anthocyan  | rubroid $C_rC_r$  |                      |
| 20        | Red stripe, stigmas yellow, anthocyan  | rubroid $C_rC_r$  | 70.5                 |
| 28X40.1   | Yellow pollen, anthocyan, pubescence, yellow anther tube, yellow ligules     | triploid ( $2n = \text{foetoid } C_rC_rE^2E^2$ ) ( $n = \text{rubroid } C_rD$ ) |                      |
| 2         | Thirteen foetida characters, nodding buds                                    | p. foetoid $C_rC_rE^2E^2$   | 24.6                 |
| 3         | Ten foetida characters, nodding buds   | funoid $C_rC_rD E^2$  | 34.2                 |
| 4         | Eight foetida characters, nodding buds                                       | p. foetoid $C_rC_rE^2E^2$   | 97.9                 |
| 28X42.1   | Anthocyan, red stripe  | (lost roots)  |                      |
| 5         | Anthocyan  | rubroid $C_rC_r$  |                      |
| 17        | Anthocyan  | rubroid $C_rC_r$  | 97.9                 |
| 18        | Anthocyan, red stripe  | rubroid $C_rC_r$  |                      |
| 21        | Anthocyan  | rubroid $C_rC_r$  |                      |
| 22        | Anthocyan, colored tips  | p. rubroid $C_rC_r$   | 97.9                 |
| 23        | Anthocyan, colored tips only, narrow head                                    | p. rubroid $C_rC_r$   |                      |
| 24        | An <sup>h</sup> ocyan  | rubroid $C_rC_r$  |                      |
| 25        | Anthocyan, colored tips  | p. rubroid $C_rC_r$   | 94.1                 |
| 27        | Anthocyan  | (lost roots)  | 97.7                 |
| 29Z 6     | Fourteen foetida characters, nodding buds                                    | foetoid $C_rC_rE^2E^2$  | 57.3                 |
| 28X43.1   | Colored tips, nodding buds   | funoid $C_rC_rD E^2$  | 57.3                 |
| 28X44.1   | Yellow anther tube, erect buds, yellow ligules, pubescence                   | foetoid $C_rC_rE^2E^2$  |                      |

The relevant data from the twenty-eight plants constituting this generation are included in table 2, where the plants comprising the six cultures represented are listed with their *foetida* characters, *foetida* chromosomes, and the percentage of good pollen grains.

The two cultures in which there is a fair representation of the selfed generations are 28.001 and 28X42. Their examination may provide some information concerning the manner of distribution of the heteromorphic pairs under study.

It will be seen from the above table that there are eleven rubroid ( $C_rC_r$ ) plants, five pseudo-rubroid ( $C_rC_f$ ), and five pseudo-rubroid ( $C_fC_f$ ) plants, one of which was doubtful.

The agreement between the observed and calculated distribution is:

|                     | $C_rC_r$ | $C_rC_f$  | $C_fC_f$ |
|---------------------|----------|-----------|----------|
| Calculated .. . . . | 13.125   | 5.25      | 2.625    |
| Observed .. . . .   | 11       | 5         | 5        |
| Deviation.....      |          | $\pm .25$ |          |

The probable error ( $0.6745 \sqrt{p \cdot q \cdot n}$ ) for such a population is 1.35 and the probability of such a deviation being due to chance is about 90 per cent on a basis of three homomorphic to one heteromorphic.

Such data for the pair  $DE^2$  will be obtained from an inspection of all twenty-eight plants, and when analyzed in a similar manner the distribution is:

|                     | $D D$ | $D E^2$   | $E^2E^2$ |
|---------------------|-------|-----------|----------|
| Calculated .. . . . | 17.5  | 7.0       | 3.5      |
| Observed .. . . .   | 21    | 3.0       | 4.0      |
| Deviation.....      |       | $\pm 4.0$ |          |

In this case the probable error is 1.55 and the probability is only 8.9 in 100; but, as previously pointed out, the disparity in numbers between the six cultures is not expected to furnish critical data.

On plate 10 there are three photographs, showing growth habits of plants representing cultures (a) 28X42, (b) 29Z6, and (c) 28X40, together with *rubra* and a *rhoeadifolia* derivative quite similar to the parent strain. Notice in *a* the almost perfect resemblance of 28X42 (29Z8) to *rubra* (R 1110), and the highly variable expression of the

three plants representing 28X42 in photograph (c). One of them is almost identical with *rhoeadifolia*, while the other two obviously show intermediacy.

Therefore, such critical evidence as exists points to the random (a) segregation of chromosomes, (b) formation of gametes, and (c) assortment of phenotypes.

## CORRELATION OF CHROMOSOMES WITH CHARACTERS

A closer inspection of the population 28.001 shows that among the six so-called rubroid ( $C_rC_rDD$ ) plants there is quite a range of *foetida* characters in evidence. Plant 19 exhibits but one *foetida* character, while the others show more, and plant 12 shows six of them. Yet in the cytological examination of these six plants it was impossible to distinguish any *foetida* chromosome. This means that the isomorphic pairs, A, B, and E, comprise some *foetida* chromosomes, or there has been some interspecific crossing over, or both.

With regard to the seven pseudo-ruboid plants, however, two of which have  $C_rC_r$  and five  $C_rC_r$ , it will be noticed that those having  $C_rC_r$  have the purple on the anther tubes restricted to the tips, whereas those having  $C_rC_r$  have, among their *foetida* characters, yellow anther tubes, as well as erect buds. The  $C_rC_r$  plants show entirely purple anther tubes and nodding buds. Plant 28X44.1 ( $C_rC_rE^2E^2$ ) agrees with the five  $C_rC_r$  plants of 28.001 in having yellow anther tubes and erect buds.

The ten plants constituting culture 28X42 likewise show assortment of the  $C_r$  chromosome, three plants being classified as  $C_rC_rDD$  and the remainder as  $C_rC_rDD$ . Those with  $C_rC_rDD$  have restricted color on the anther tubes, whereas those having  $C_rC_rDD$  are solid colored. All plants have nodding buds.

Photographs of three plants of 28X42, figure a, plate 10, illustrate the predominance of *rubra* characters, compared with the *rubra* parent shown alongside. It will be noticed from table 2 that very few other *foetida* characters occur in any of the plants, and the presence of anthocyanin, common to all, is doubtless contributed from both species, although it is seldom seen in pure *rubra* plants.

However, the fact that all nineteen plants of cultures 28.001 and 28X42 have two *rubra* D-chromosomes, may raise some doubts concerning the proposal for homologizing  $C_rC_r$  instead of accepting

Navashin's homology of  $DC_r$ . Such doubts are dispelled by the fact that plant 28X44.1 ( $C_rC_rE^2E^2$ ) and the three diploid plants of  $F_2$  in Cross II, see table 4, all confirm the above evidence and in addition all lack any D-chromosomes. This proves that  $C_r$  alone contains the factor which would disturb yellow anther tube pigmentation, causing purple anther tips when together with  $C_r$ . Even though the gross morphology of these plants suggests *foetida*, all of them agree in possessing this one *rubra* character, as well as the  $C_r$  and not the D.

Furthermore, the three triploids of Z14 all have the  $C_r$  and colored anther tube tips, whereas Z13.2 (also  $3n$ ) was classed as having entirely purple anthers tubes and since its extra set was  $C_rD$ , this is to be expected. The question of bud position does not enter in Cross II because the strain of *foetida typica* which was used in this cross has only nodding buds.

Returning again to Cross I, as shown in table 2, there are four plants in the population 28X40, the gross morphology of which, as well as the somatic garnitures, is predominately *foetida*. 28X40.1 is a triploid, and the somatic plate discloses a situation which can only be interpreted on the assumption that the diploid gamete was foetoid ( $C_rC_rE^2E^2$ ) and the haploid was rubroid ( $C_rD$ ). But for the non-reduction of one of the gametes this plant would have been a funoid ( $C_rC_rDE^2$ ) and has been so classified in table 2. The plant possessed yellow anthers and nodding buds. The remaining three plants of this culture all agree in having  $C_rC_r$ . Morphologically they have nodding buds as expected, but also yellow anther tubes, not expected from the foregoing discussion.

Of the three remaining plants of this backcross selfed generation, 28X44.1 is  $C_rC_r$ , and has yellow anther tubes as well as erect buds. 29Z6 being also  $C_rC_r$  agrees in having yellow anther tubes but disagrees in having nodding buds, to be seen from photograph *b*, plate 10. Plant 28X43 is  $C_rC_r$  and shows nodding buds and colored tips, as expected for a funoid plant.

According to the facts, the hypothesis may be advanced: that  $C_rC_r$  gives purple anther tubes and nodding buds,  $C_rC_r$  gives purple tipped yellow anther tubes and nodding buds, and that  $C_rC_r$  gives entirely yellow anther tubes and erect buds, barring the incidence of interspecific crossing over. We then have perfect agreement in thirteen plants from five cultures, but disagreement in four cases, representing but two cultures, and these cultures are not well represented numerically.

The discrepancy in the matter of yellow anther tubes for  $C_rC_r$  in the three plants of 28X40 might easily have resulted from an error in classification, but there is an alternative explanation in view of the fact that *Crepis* workers recognize that in some *rubra* strains there is variability in the intensity and extent of the purple pigmentation on the anther tube.

In the case of bud position the discrepancy is still more readily disposed of, since erect buds are dominant in intraspecific crosses in *C. foetida*, although recessive in all interspecific crosses, and the parental strain 1539 is known to be heterozygous for the character. Doubtless the one plant used as parent in Cross I was heterozygous. In all cases nodding buds are dominant over erect buds in derivatives.

The possibility that these exceptions are due to interspecific crossing over is to be further investigated with a larger  $F_2$ . If only the three phenotypic classes found this year are then obtained there will be indication of no crossing over, but if the six possible phenotypes are obtained, then the linkage value can be computed from the frequency of the yellow-anther-tube erect-bud class, since it will be the double recessive class. The situation may be represented as below:

|                 |                 |
|-----------------|-----------------|
| E, nodding buds | e, erect buds   |
| P, purple tubes | p, yellow tubes |

$F_1$        $\frac{EP}{ep}$

Expected types in  $F_2$ :

EP, nodding buds, purple tubes;  
 EPp, nodding buds, purple tipped tubes;  
 Ep, nodding buds, yellow tubes;  
 eP, erect buds, purple tubes;  
 ePp, erect buds, purple tipped tubes;  
 ep, erect buds, yellow tubes.

Regardless of any question as to whether or not interspecific crossing over has or will occur, the situation with respect to a correlation between the character pairs and the chromosome pairs may be summarized as follows: agreements with the hypothesis are consistent in the two cultures having the most significance, i.e., 28.001 and 28X42, whereas the discrepancies are fairly easily disposed of. Therefore, chromosome C, whether  $C_r$  or  $C_r$ , may be considered to contain the factors conditioning coloring of the anther tubes and position of the buds before anthesis. Furthermore, the fact that the three diploid members of the  $F_2$  population of Cross II agree with the hypothesis, and at the same time lack the *rubra* chromosome D, proves that  $D_r$  cannot be homologous with  $C_r$ , as Navashin tentatively assumed in 1925.



## THE $F_1$ GENERATION

In an attempt to duplicate some of the earlier steps of this cross, a strain of *foetida typica* was crossed with a white-flowered strain of *rubra*, both similar to the parents employed by Navashin in Cross II. By so doing it was hoped to avoid the sterility introduced into Cross I by the use of *foetida rhoeadifolia*. One  $F_1$  individual was secured from this cross. A photograph of it, showing the intermediate expression of habit and size between the adjacent parents is illustrated in plate 9. Its somatic garniture is illustrated in figure 4a, showing the convenient distinctions between the two parents set up by the two heteromorphic pairs  $D/E^2$  and  $C_r/C_r$ .

Owing to a limited number of flower-heads, and the desirability of utilizing as many as possible in the production of backcrosses and  $F_2$  individuals, only a few buds were fixed for a study of meiosis. These were stained for temporary aceto-carmin examination. In preparation of *Compositae* mounts by this method such a large number of PMC's are eliminated in removal of sporophytic tissue that usually relatively few division figures are retained. Furthermore, the chromosomes are more swollen and larger than those of material fixed for permanent mounting.

The one slide obtained confirmed the fact that five bivalents occur in  $F_1$ . See figure 4b. Nothing was then learned of the frequency of occurrence of complete pairing, as studied by Babcock and J. Clausen (1928) in three hybrids between four-paired species, but deductions based on the percentage of good pollen grains place the figure around 2.7 per cent, as shown in table 4.

It would be highly desirable to know something of the frequency of occurrence of unreduced gametes in  $F_1$ . An estimate may be made indirectly from  $F_2$  data. From the nine plants of this population (see table 4) the eighteen parental gametes consisted of seven diploid and eleven haploid gametes, or approximately 39 per cent unreduced, and 61 per cent reduced.

## MEIOSIS IN A FUNOID PLANT

Since the chief interest in Cross I is attached to a study of external morphology and chromosome individuality, few of the hybrid derivatives needed to be studied for the reduction division. The fact that

the four plants from 28X40 showed more than the usual number of *foetida* characters, however, suggested the advisability of such study here.



Fig. 5. Drawings from a funoid derivative ( $C_r C_r DE^2$ ). *a*, somatic garniture; *b*, IM, showing five bivalents; *c*, non-conjunction in one pair; *d*, *e*, tetrad stages.

Figure 5*a*, an illustration of what is meant in the preceding discussion by a funoid garniture ( $C_r C_r DE^2$ ), admirably shows a plate apparently identical with the garniture of a true  $F_1$  plate as illustrated in figure 4*a*. In the two late metaphases, shown in figure 5*b*

and *c*, are shown situations that might be expected from our previous knowledge of occasional complete pairing in  $F_1$ , together with irregularities, in this case non-conjunction of two univalents. From figure 5*d* and *e*, however, the situation is seen to be still more complex than would be expected from a single pair of non-conjugating chromosomes.

In table 2 it is seen that the *percentage of good pollen* for this plant is 34.2 per cent. In table 4, as discussed above, it is shown that for  $F_1$  plants the *percentage of good pollen* is only 2.7 per cent. Now, obviously in the latter case we have the maximum amount of heterogeneity to be expected in hybrid derivatives; therefore, with eight times as many well stained pollen grains, we may conclude that 28X40.3 contains true homology in some of the three more or less isomorphic pairs of chromosomes (i.e., A's, B's and E's).

From the evidence thus far considered in the backcross to *rubra* it is clear that study of a larger backcross generation, as well as of a larger selfed backcross generation, will prove invaluable in elucidating some perplexing problems of interspecific hybridization, e.g., character-chromosome correlations, method of chromosome distribution, and viability of gamete types.

### THE BACKCROSS TO *FOETIDA*

Selfed backcrosses to the *foetida* parent proved so sterile that only seven achenes were viable. Had the resultant population been of sufficient size it would have been desirable to have ordered the investigations along the line of the backcross to *rubra*, with the exception of attempted correlation of *rubra* chromosomes with *rubra* characters. Inability to conduct this phase of the problem further emphasizes the inconvenience of having used *rhoeadifolia* instead of *foetida typica*, because of the sterility ensuing from abnormal reduction.

A glance at table 3 shows that all the plants of the backcross generation to *foetida*, with the exception of 28Z9.2 and Z9.3, exhibited a foetoid garniture and resembled *foetida* in morphology. These exceptions have nodding buds and are both  $C_rC_r$ , again according to expectation (see pl. 9, fig. *b*).

Notes taken during morphological examination mention only yellow anther tubes in all cases, but it is entirely possible that colored tips in the pseudo-foetoid plants were present and overlooked. It will be noticed that all  $C_rC_r$  plants have erect buds. The data gathered in 1928 were not as complete as those gathered later.

The predominant type of garniture represented in this selfed backcross is foetoid, which is to be expected from the predominance of rubroid types in the reciprocal backcross.

The three plants growing in 1929 are shown in plate 9, figure *b*, where the *rhoeadifolia* habit of growth is evident.

TABLE 3

RUBRA CHARACTERS AMONG PLANTS OF THE ONE SELFED FOETIDA BACKCROSS

| Plant   | Rubra characters                               | Somatic type        | Per cent good pollen |
|---------|--|---------------------|----------------------|
| 28X45.1 | —  | foetoid $C_rC_r$    |                      |
| 28Z 9.1 | No red stripe, erect buds                      | foetoid $C_rC_r$    |                      |
| 2       | Nodding buds                                   | p. foetoid $C_rC_r$ | 49.3                 |
| 3       | Nodding buds                                   | p. foetoid $C_rC_r$ | 55.3                 |
| 29Z 9.1 | Open all day, medium height, erect             | foetoid $C_rC_r$    | 93.8                 |
| 2       | Open all day, short stature, broad head, erect | foetoid $C_rC_r$    | 46.2                 |
| 3       | Open all day, broad head, erect                | foetoid $C_rC_r$    | 60.8                 |

## CROSS II

There were about six  $F_1$  achenes of Cross II brought to Berkeley from Moscow by Dr. Navashin. One of these was transported to the Genetics Division garden at Palo Alto. Several heads of this plant were bagged and at the proper time all normal looking achenes were gathered and labeled. The plant had been isolated from other *Crepis* species, obviating the chances of crossing in the open pollinated heads. In the spring of 1929 the seed from this selfed plant was sown at Berkeley, and all plants that germinated reached maturity; three from the open heads and six from the bagged heads. Examination of root tip material of these nine plants revealed the situation shown in table 4.

There are three diploids, all of the pseudo-foetoid ( $C_rC_r$ ) type, five triploids with constitution denoting an unreduced  $F_1$  gamete plus: one rubroid, two foetoids, and two pseudo-foetoids, respectively.

Photographs of these nine plants are shown on plate 11 where an excellent idea of the gross morphology of each plant may be formed. Notice especially the resemblance of the amphidiploid to the  $F_1$  branching habit illustrated in plate 9, figure 1; also how the triploids derive their gross morphology from the parent represented by the assumed extra set. For instance 13.2 is shorter in stature and has much broader heads than 13.3, the former having a rubroid set

extra, the latter a foetoid set extra. All six plants of 29Z14 appear to resemble *foetida* uniformly, yet three of them are  $2n$ , and three are  $3n$ . All six, however, have purple tips on their anther tubes, a *rubra* character. All six have the  $C_r$  and only three have  $D$ , thus the  $D$  appears to have no effect in conditioning the color of anther tubes, as previously noted.

TABLE 4  
F<sub>2</sub> GENERATION OF CROSS II

| Plant No | Rubra characters   | Somatic type                    | Per cent good pollen |
|----------|--|---------------------------------|----------------------|
| 29 Z13.1 | Intermediate   | amphidiploid                    | 34.5                 |
| 2        | Characters mostly <i>rubra</i> , purple tips                             | $3n$ , ex. set $C_rD$           | 20.0                 |
| 3        | Characters mostly <i>foetida</i> , yellow anther tubes                   | $3n$ , ex. set $C_rE^2$         | 36.5                 |
| 29X14.1  | Short life, white ligules, purple tips, outer bracts <i>rubra</i> shaped | $2n$ , p. foetoid $C_rC_r$      | 20.2                 |
| 2        | Characters mainly <i>foetida</i>   | $3n$ , ex. set $C_rE^2$         | 29.7                 |
| 3        | Characters mainly <i>foetida</i>   | $3n$ , ex. set $C_rE^2$         | 17.9                 |
| 4        | Characters mainly <i>foetida</i>   | $3n$ , ex. set foetoid $C_rE^2$ | 9.0                  |
| 5        | Purple tips, <i>rubra</i> outer bracts, bracts glabrous                  | $2n$ , p. foetoid $C_rC_r$      | 8.3                  |
| 6        | Purple tips  | $2n$ , p. foetoid $C_rC_r$      | 38.2                 |
|          | <i>Rubra</i> strain 1110   |                                 | 96.6                 |
|          | <i>Foetida typica</i> (strain similar to that of Cross II)               |                                 | 95.4                 |
|          | $F_1$ ( <i>foetida typica</i> × <i>rubra alba</i> )                      |                                 | 2.7                  |

From these data it is difficult to say in the triploids whether it is the megaspore or microspore that has been unreduced; but from the occurrence of unreduced gametes in 39 per cent of the cases, and the chance meeting of two of them to produce an amphidiploid, there can be little question that both gametes on the same plant are affected. Figure 6a excellently depicts the complete diploid complements of both parents in the amphidiploid.

From table 4 it is seen that this plant has only 34.5 per cent good pollen, almost identical with the percentage found by Hollingshead (1930) in her *capillaris-tectorum* amphidiploid. One immediately suspects from this that irregularities of the reduction division are responsible. Accordingly an investigation was begun into its meiotic behavior.



Fig. 6. Preparations from an amphidiploid. *a*, somatic garniture; *b*, *c*, diaphases showing five and seven units; *d*, *e*, *f*, IM showing five, six, and seven units.

Paraffine sectioned buds of *Compositae* material are especially favorable for such a study and disclose a wide range of conditions on adjacent concentric circles of florets. The outer row may have pollen mother cells in the tetrad stage, the second row, or only part of it, may have second division figures, while the remainder of that row, and part of the third are in first metaphase or earlier. At the same time in the center of the bud some of the tissue may not yet have reached the gametophyte stage. Consequently when first division figures are present practically all information desired is available, if the fixation is good. This situation is quite unlike that found when aceto-carminic mounts are used, for in such a case most of the PMC's would remain within the anther tubes.

From a study of figures 6 and 7 it is evident that quadrivalents are frequent. In the plates illustrated it will be seen that cell complements range from five to thirteen units at the first metaphase. Such variability in conjugation would naturally result in irregularities at the end of the second division, shown in figure 7c.

When one finds five bivalents in  $F_1$  it is not surprising that five quadrivalents should occasionally be found in a  $4n$  individual, and the fact that intergrading types of conjugation occur, with consequent elimination of microcytes and micronuclei, would certainly account for the major portion of the 65.5 per cent non-staining pollen grains obtained in aceto-carminic determinations. However, a sufficient number of viable gametes are produced to insure a fair degree of fertility upon selfing. From twenty-two heads fifty-five achenes were gathered. This shows a higher degree of fertility than in the best diploid, 29Z14.6, which produced fifty achenes from about forty-two heads.

Evidently when the phenomenon of amphidiploidy occurs in more or less closely related species the occasional formation of quadrivalents (in this instance, at least), and their subsequent random assortment, so upsets meiotic division that irregularities in the production of gametes operate to the disadvantage of such hybrids, as contrasted with hybrids between more distantly related species in which no pairing at all occurs, e.g., *Raphanus-Brassica*, *Nicotiana bigelovii-N. glutinosa*.

Further studies of Cross II, especially the progeny of the amphidiploid and the two fertile diploids, will be reported in a subsequent paper.

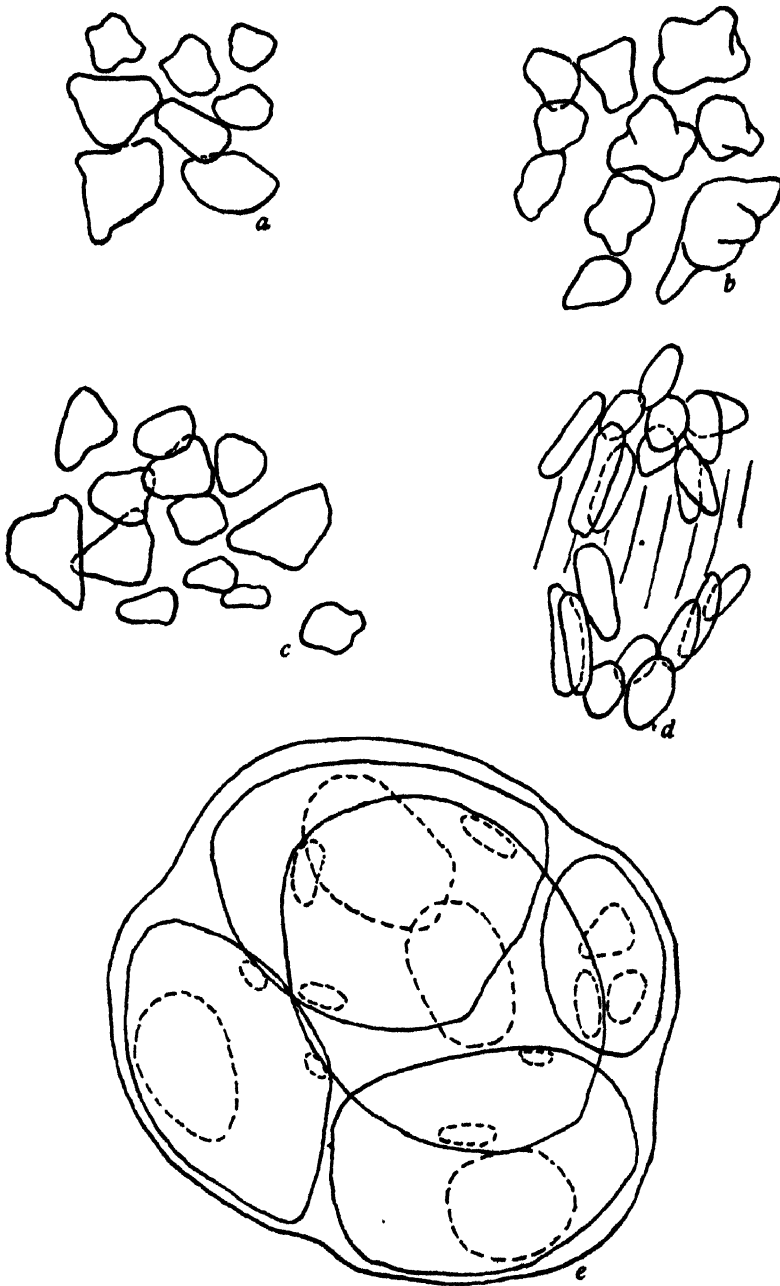


Fig. 7. From the same amphidiploid as in figure 6. *a, b, c*, IM showing eight, ten, and thirteen units; *d*, IA, showing nine to eleven univalents; *e*, tetrad stage, with microcytes.



## STERILITY AND VIABILITY

Use of the *percentage of good pollen* as an indication of fertility was not attempted generally until 1929. Therefore, populations grown in 1928 show few data on this important point. Glancing over the column per cent of good pollen in tables 2, 3, and 4, it is seen that if cultures are considered as units a fairly consistent range of fluctuation occurs. In culture 28X42 the percentage of good pollen in the five members so tested is uniform and high; in culture 28X40, the two members tested are in agreement; in culture 29Z12, all the members tested are uniform and low. The seven members representing the backcross to *foetida* likewise show some degree of uniformity, with one exception. In view of this fact it may be considered that the two determinations of 70.5 per cent and 82.9 per cent good pollen in cultures 28.001, would roughly represent the degree to be found there had more material been studied.

Such being the case it is seen that if this character be considered a measure of fertility, then those combinations representing the maximum degree of heterozygosis (as in  $F_1$ , or populations where a large number of *foetida* characters are encountered in the backcross to *rubra*), will exhibit the lowest percentages of good pollen, as tables 2, 3, and 4 show.

On the other hand, environmental factors exert a considerable rôle in sterility, as seen by the fact that no seed at all was set from culture 28.001 in September, where the determinations of good pollen were high; whereas in late spring and summer, viable seed is obtained from  $F_1$  plants, with but 2.7 per cent good pollen.

From table 4 it will be seen that the last two plants in culture 29Z14, both diploids, exhibit very low percentages of good pollen. Yet both these plants were fertile, producing twenty and fifty achenes respectively, a high proportion of which have germinated. The only other plant in this population to set any achenes was the triploid Z13.3, which produced only two.

In a study of meiosis in some *Crepis* species and hybrids, Babcock and J. Clausen (1929) show that the closer the phylogenetic relationship between the species the greater the degree of fertility and the higher the percentage of good pollen grains in the hybrids. Thus, in more distantly related species, even though their respective chromo-

somes may agree in size and shape, their genetic identity has been so altered in the course of time that homology of chromosomes is at a minimum.

Hollingshead (1930) shows that even in triploids of *C. capillaris*  $\times$  *C. tectorum*, where the former is represented twice in the somatic garniture, the percentage of good pollen grains is much higher than in  $F_1$ , and undoubtedly the degree of homology in such a triploid is higher than in an  $F_1$  diploid.

In view of these facts it may be said that percentage of good pollen grains is an indication of homology of chromosomes, but affords little idea of the proportion of viable seed to be expected.

### ACKNOWLEDGMENT

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### SUMMARY AND CONCLUSION

There are two subspecific forms of *C. foetida* used in this study, *rhoeadifolia* and *typica*, and but one of *C. rubra*. All have five pairs of chromosomes, of which three pairs are obviously homologous in somatic metaphase plates, whereas the homology of the two remaining pairs, which are known to conjugate in  $F_1$ , has been inferred from the cytological and genetic evidence.

Of the sixteen morphological character pairs used in genetic analysis, two have been assigned to the interspecific homologues designated  $C_rC_r$ .

The character pairs, purple-yellow anther tubes and nodding-erect buds before anthesis, the former of each pair from *rubra*, the latter from *foetida rhoeadifolia*, are definitely correlated with the chromosome pair  $C_rC_r$ , as used in homologizing the haploid sets of these two species.

$C_rC_r$ , purple anther tubes, nodding buds;

‘  $C_rC_r$ , purple tipped yellow anther tubes, nodding buds;

$C_rC_r$ , yellow anther tubes, erect buds.

*C. foetida rhoeadifolia* is much less fertile than *C. foetida typica*. Somatic garnitures of the strain 1539 of the former subspecies exhibit

a difference in the shape of the satellites attached to the chromosomes designated C. Cytological preparations of meiotic divisions indicate failure of one pair of chromosomes to conjugate in metaphases, with subsequent elimination of one pair at the conclusion of the first division. It is suggested that the irregular meiotic pair may be identical with the non-uniform somatic pair, and that some of the lessened fertility of *rhoeadifolia* in nature is due to partial loss of genetic homology in the pair.

The fact that no aneuploid individuals have thus far issued from derivatives of the cross *rubra* × *foetida* indicates that gametes lacking entire sets of five or ten chromosomes are mostly non-viable. So far as ascertainable, no other chromosome combinations are non-viable.

Cultures issuing from single self-pollinations agree rather closely in (a) percentage of good pollen gains, and (b) the similarity of somatic garniture types, but not in the degree of fertility; therefore, percentage of good pollen grains is useful as an index of chromosome homology, but not degree of fertility.

In selfing the backcross of  $F_1$  to *rubra* there was obtained among the better numerically represented cultures, a sufficient range in chromosome assortment from the two species, so that upon the basis of random distribution of the two heteromorphic pairs  $C_rC_t$  and  $DE^2$ , a close approximation was obtained of the expected with the observed assortment.

Meiosis of plants showing maximum degrees of heterozygosis, i.e., true  $F_1$  and funoid types ( $C_rC_tDE^2$ ), exhibit instances of complete pairing. An amphidiploid plant of this cross exhibited instances of complete quadrivalence. The population from which the amphidiploid arose indicated that unreduced gametes existed in both sexes, and that among the nine ensuing  $F_2$  plants there were one amphidiploid, five triploids, and three diploids; therefore a ratio of eleven reduced to seven unreduced gametes.

Although the two species *rubra* and *foetida typica* show a considerable number of distinct morphological differences, there is sufficiently close relationship in the amphidiploid that homologous chromosomes from the two species tend to form quadrivalents in varying frequency during meiosis, and the ensuing irregularities at reduction cause a diminution in fertility.

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## EXPLANATION OF PLATES

PLATE 9

a. Illustrations of growth types of (left to right): 1, *C. foetida typica*; 2,  $F_1$ ; 3, *C. rubra*; 4, *C. foetida rhoeadifolia* (derivative).

b. Three representatives of the population obtained by selfing the progeny of the backcross,  $F_1 \times foetida\ rhoeadifolia$ .



a



b

## PLATE 10

Representatives of generation of  $F_1$  backcrossed to *rubra*, selfed.

a. Plant at left, *C. rubra*, strain 1110; three plants right, derivatives of the culture 28X42, showing a close resemblance to *rubra*.

b. Left plant, *C. rubra*; center, 29Z6, a foetoid type with somatic garniture indistinguishable from *foetida* but exhibiting many *rubra* characters; right, *foetida rhoeadifolia* derivative.

c. Three plants of the culture 28X40, showing morphological gradations between the two parents: left, p. foetoid (28X40.2); center, funoid 28X40.3; right, p. foetoid (28X40.4), showing closest resemblance in branching habit to *foetida*.





a



b



c

## PLATE 11

F<sub>2</sub> generation of *C. rubra* × *C. foetida typica*.

a. Left, amphidiploid, already past its first flowering cycle—three other cycles followed and its ultimate height approached the central plant; center, a triploid, with extra set in somatic garniture apparently *rubra* (morphology resembles *rubra* more than the amphidiploid or the plant at the right); right, a triploid with extra somatic set apparently *foetida*, and exhibiting more *foetida* characters than the other two plants (two achenes obtained).

b. 1, a sterile diploid of somatic type C.CrE'E'; 2, 3, and 4, triploids with extra sets resembling *foetida*; 5 and 6, fertile diploids, both with somatic garnitures of the type of plant 1.



a



b

**SPONTANEOUS CHROMOSOME ALTERATIONS  
IN CREPIS TECTORUM L.**

**AND**

**CHROMATIN MASS AND CELL VOLUME  
IN RELATED SPECIES**

**BY**

**M. NAVASHIN**

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# SPONTANEOUS CHROMOSOME ALTERATIONS IN *CREPIS TECTORUM* L.

BY  
M. NAVASHIN\*

During the summer of 1930 many progenies derived from *Crepis tectorum* plants possessing aberrant chromosome sets were grown for the purpose of studying the transmission of chromosomal abnormalities. It was found in the great majority of cases, in full accord with expectation, that chromosomal abnormalities were transmitted as such to a certain part of the offspring. Thus, for instance, simple trisomies threw identical trisomies in proportions which varied according to the kind of the extra chromosome and according to the individual. Individuals possessing a typical chromosome structure (for instance, translocated chromosomes) transmitted their abnormal chromosomes to a certain percentage of their offspring. In three cases, however, the behavior of the tested individuals was entirely different. They produced, indeed, among their progeny not only the expected chromosomal abnormalities, i.e., aberrations identical with those which were characteristic of them, but there appeared also in the immediate offspring some entirely new chromosomal alterations, and, furthermore, these occurred in unusually high numbers. In the following pages will be given a short discussion of these peculiar cases observed among progenies 30.503, 30.511, and 30.515.

Progeny 30.503 was grown from open pollinated seed collected from a simple trisomic. It consisted of seven plants from which two were selected for cytological investigation because of their somewhat abnormal appearance. One of the two plants died early in development; the other was subjected to cytological investigation.

From the seventeen root-tips investigated thirteen proved to be entirely normal. Four of them uniformly contained an abnormal chromosome complement shown in figure 1, *d*. From an inspection of this figure one can see that the alteration involves two chromosomes, namely, the two A-chromosomes (see, for a normal complement of

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*Crepis tectorum*, fig. 1, *h*) which at first sight seem to be entirely lacking. Closer examination and comparison with the normal complement make it possible to give to this phenomenon an adequate interpretation. It is easy to see that two conspicuous structures have replaced the two A-chromosomes in this figure and that the large V-shaped chromosome and the other very short one are simply products of rearrangement of the material which normally composes the two A-chromosomes. Obviously, the proximal fragment of one of the A-chromosomes became detached as a diminished autonomous chromosome; the rest of the same A-chromosome (its distal fragment) has fused with the proximal end of the other, otherwise unaltered A-chromosome, making its smaller arm correspondingly longer and thus producing a large V-shaped structure.

The upper parts of the plant in question not having been investigated, nothing can be said about their chromosomal constitution. It appears probable, however, that certain shoots possessed some chromosomal abnormalities since they displayed a very low fertility. The latter circumstance would be expected in case the above described chromosomal alteration was present also in spore mother cells.

Progeny 30.511 was derived from open pollinated seed produced by a simple trisomic. From the total of forty plants, five appeared to be more or less abnormal and the remaining thirty-five were discarded. Cytological investigation of the root-tips of these morphologically aberrant individuals showed that two of them were simple trisomies of the triple-B type (like the parental plant), two were apparently normal, and one was a chromosomal chimera. Among many root-tips there was only one cytologically abnormal; it was uniformly altered in all its cells in a most conspicuous way. The alteration in this case involved the two D-chromosomes (fig. 1, *a*, *b*, and *c*). As may be easily deduced, the proximal fragment of one of the D-chromosomes became an autonomous small satellited chromosome, the distal portion of the same chromosome being permanently attached to the satellite of the second (otherwise unaltered) D-chromosome. The resulting alteration of the chromosome complement was thus expressed by the presence of one minute satellited chromosome and another very large one distinguished by its unusually large cylindrical satellite.

This particular plant did not show any abnormalities as to its fertility, etc., so that it seemed rather probable that this alteration was localized in a small part of the root system.

Progeny 30.515 came from open pollinated seed yielded by a chromosomally aberrant plant which possessed a very small spherical fragment in addition to its otherwise apparently normal chromosome complement. Among the total of twenty-four plants, five were selected for cytological investigation since they were distinguished by various morphological peculiarities. Only one of them (plant 3) was probably normal in its chromosome complement, but owing to the lack of good roots, this could not be established with full certainty. The remaining four plants were all chromosomally abnormal, and moreover, each of them in a different way.

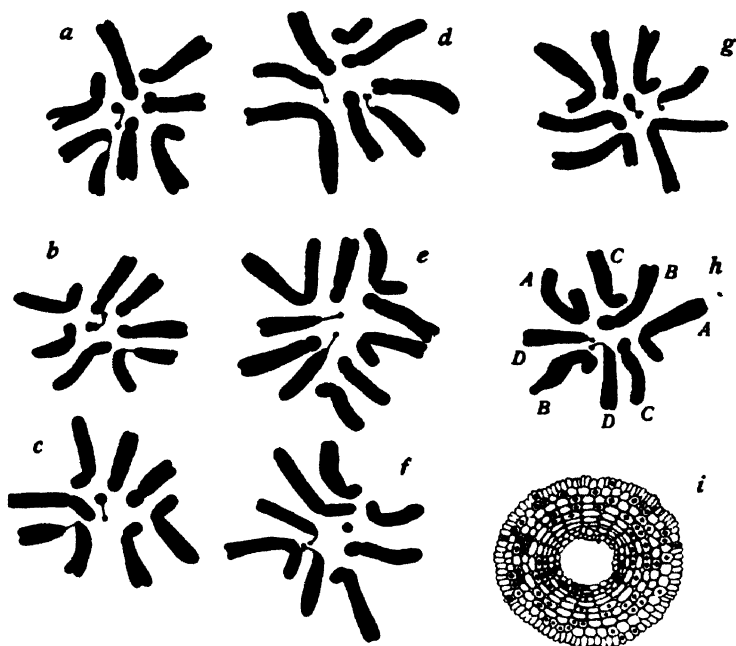


Fig. 1. Somatic chromosomes from the root-tips of altered individuals of *Crepis tectorum*.

*a, b, c*, three metaphases from the single altered root of the plant 30.511-2. One of the two D-chromosomes was fragmented; its proximal fragment constitutes a small satellited chromosome, the distal portion being permanently fused with the satellite of the other, otherwise unaltered D-chromosome.

*d*, plant 30.503-2. One of the two A chromosomes fragmented; its proximal fragment functions as a small autonomous chromosome; the distal portion is attached to the proximal end of the other A-chromosome thus forming a large V-shaped structure.

*e, f, g*, different plants from progeny 30.515 showing various chromosomal alterations.

*h*, a normal chromosomal complement from the chimera root belonging to plant 30.515-2 (cf. fig. 1, *g*).

*i*, diagram showing the distribution of altered and normal mitoses in the root of plant 30.515-2 (cf. fig. 1, *g*). Crosses indicate altered cells, discelets, normal cells.

See also the text. Magnification 1700 diameters, except diagram *i* which is drawn to a much smaller scale.

Plant 1 was a simple triplo-A trisomic, all its roots uniformly possessing an extra A-chromosome.

In plant 2 one root out of fourteen was partly altered in its chromosomes. There appeared a sector forming about 75 per cent of the whole root in which the proximal fragment of one of the two D-chromosomes became autonomous (as in the case of progeny 30.503), the distal portion of the same chromosome being fused with the proximal end of one of the B-chromosomes, thus forming again a large V-shaped structure (fig. 1, *g* and *i*).

Plant 4 (fig. 1, *c*) was uniformly altered in all its roots. In this case there was present one C-chromosome extra, and, in addition, a large V-shaped structure apparently derived from fusion, as in the instances described above.

Finally, plant 5 displayed the same peculiarity as the parental individual, namely, one of its roots contained in all its cells a very small spherical fragment (fig. 1, *f*).

None of the above chromosomal abnormalities seemed to influence the mitotic process, nor did they seem to affect in the slightest degree the cell sizes or the vitality of the cells, etc. The latter was especially clear in the case of plant 30.515-2 where the sector of the root containing the altered chromosomes did not differ in regard to its cell divisions, cell size, shape, etc., from the chromosomally normal part of the root (cf. fig. 1, *i*). It should be concluded, therefore, that even such profound rearrangements of the chromosome material as those described above have no physiological effect whatever, at least so far as roots are concerned. One can hardly doubt, moreover, that they also produce no effect upon any other somatic tissue, at least in the earlier stages of its development.

The observations reported here not only add some new cases of chromosomal alterations to those previously known to occur in *Crepis* spontaneously (M. Navashin, 1926) or after the x-ray treatment (M. Navashin, 1931), but they also throw some light upon the conditions under which such chromosomal alterations are most likely to occur. First, it may easily be seen that in the majority of cases they originate in somatic mitoses and sometimes very late in ontogeny because the majority of individuals developing them are chimeras. Secondly, it seems probable that there exists some peculiar condition in certain individuals, which makes their chromosome structure and the chromosomal distribution labile and subject to frequent alterations in various ways. It may be suggested that just these three individuals which

gave rise to the progenies reported above possessed some inherent and perhaps heritable instability of chromosome behavior, which could account for the fact that, of their offspring, an unusually high proportion of individuals was altered in several different ways. It is necessary to point out in this connection that the frequency of spontaneous chromosomal alterations normally does not reach even 0.1 per cent, while in the above cases the corresponding frequency was almost one hundred times as high.

While chromosomal alterations representing only rearrangements of the chromosome material (dislocations) could hardly produce any immediate visible effect, some of them would inevitably lead to changes in chromosome affinity. Thus, for example, if plant 30.515-2 (fig. 1, *g*) could transmit its chromosomal peculiarity in homozygous form, individuals could arise which would possess an entirely new mode of chromosome conjugation in meiosis. From what is known now of homozygous translocation in *Drosophila melanogaster* (Dobzhansky, 1930), such organisms can really exist and, moreover, can be fertile. If, now, such an individual should be crossed with the original normal form, the hybrid would inevitably suffer from meiotic disturbances involving the translocated chromosomes, in the case referred to above, chromosomes B and D. For the normal B-chromosome will be attracted by one part of the fusion chromosome, the D-chromosome will tend to conjugate with the other part of the fusion chromosome, and the small free fragment of the D-chromosome will be attracted by the corresponding part of the D-chromosome. In such a hybrid normal reduction division would be impossible and the homozygous form possessing the dislocated chromosomes would become more or less genetically isolated from its progenitor. Thus it seems probable that certain chromosome alterations of the type described above may play an important rôle in the initial steps of species formation.

It should be noted that alterations originating in undifferentiated sporophytic tissue would have a decided advantage over those arising during gametogenesis. For, in case the altered sporophytic tissue should produce reproductive organs, altered functional gametes might arise at once in great numbers and thus the new chromosome organization would be transmitted to the offspring more certainly than in the case of occasional formation of single aberrant germ cells during sporogenesis.

The above facts may be advantageously applied also to the explanation of some characteristic chromosome relations existing among

species. Thus, for instance, the origin of the large V-shaped chromosomes typical of many species, especially of the *pulchra*-group in *Crepis* (Babcock and Navashin, 1930) may be attributed with a fair degree of probability to fusion of the sort described above. The occurrence of very small satellited chromosomes (for instance, in *Crepis parviflora*, *loc. cit.*) may be explained as a result of fragmentation, since the D-chromosomes are especially apt to detach free proximal fragments. Finally, chromosomes with unusually large satellites, as in *Crepis setosa* with its unique satellited chromosome, could arise through translocation of a considerable chromatin portion to the satellite (cf. fig. 1, *a*, *b*, and *c*).

All these considerations are concerned with translocations or mere rearrangements, first designated dislocations (M. Navashin, 1926). If, in addition, some gain or loss of chromosome material takes place, one would deal with the change of genic balance, and, consequently, with more or less far-reaching changes in the organization of the individual bearing in its cells these new genetic conditions. It is obvious that any heterozygous dislocation would ultimately result in gain and loss of chromatin material in succeeding generations owing to segregation of chromosomes. And, if not incompatible with life, these may result in variations of evolutionary significance. For there can be hardly any doubt that the evolution of *Crepis* species was primarily based upon changes in the quantity of the material contained in the individual chromosomes (cf. Babcock and M. Navashin, 1930).

Further investigation is now in progress. Attention will be devoted chiefly to the study of the transmission of the presumed hereditary lability of chromosome behavior and to the problem of obtaining homozygous translocations.

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# CHROMATIN MASS AND CELL VOLUME IN RELATED SPECIES

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## INTRODUCTION

At the present time the existence of a parallelism between nuclear mass and morphology and external structure of organisms can be demonstrated only for polyploids. There is no such clear relationship, however, among the different species. A tetraploid species is not necessarily larger than a diploid species; moreover, instances are known where analogous changes in the chromatin mass have exactly opposite results in two different species.

Such a lack of straight parallelism between nuclear mass and morphology and external organization may be reasonably explained by secondary influences of certain genes modifying the development in such a way that the original relations become obscured. This may be observed even in some polyploids, for instance, in *Oenothera gigas nanella*, which, instead of having the giant stature typical for tetraploids, is a dwarf, owing to a specific influence of a particular gene.

In order to arrive at a clearer understanding of this problem, one must study, therefore, earlier stages of development, prior to any differentiation or mutual interaction of tissues. Such conditions are realized best in the primary meristem of a plant, where one might expect to observe the original relation between the nucleus and development, unobscured by differentiation. But because of lack of differentiation only a few characters can be studied in meristematic cells. Among these primordial characters the size of the cell may be considered the most important; for it can be accurately measured and, moreover, it undoubtedly reflects the fundamental property of the cell, the ability of synthesizing a definite amount of protoplasmic substance.



The cell size was selected, therefore, for the present investigation in the hope that it may give a clue to some understanding of the significance of specific differences in nuclear organization.

The present investigation was conducted in the Division of Genetics of the University of California under a fellowship of the International Education Board. The writer acknowledges with gratitude the many helpful suggestions of Professor E. B. Babcock and Professor R. E. Clausen during the course of the work.

## MATERIAL AND METHOD

Primary meristem of root tips was chosen as material for the present investigation. Only primary roots of very young seedlings were used, since it was found that they show less variation in cell size than the adult roots. The seeds of different *Crepis* species were planted in sand and germinated under conditions of moisture and temperature held as constant as was possible under the circumstances. Immediately after the cotyledons of the seedlings had spread, the root tips were fixed in S. Navashin's fixative (chromic acid, acetic acid, and formalin); special care was taken to perform all the following procedures in a uniform manner in order to prevent errors due to unequal shrinkage of different lots of material, etc. The sections were uniformly 10 microns thick; they were stained in iron haematoxylin and mounted in Canada balsam.

Thirteen *Crepis* species belonging to different sections of the genus were selected for the investigation; in their total chromosome length they ranged from 42 to 112.1 relative units.

After several preliminary trials, cells of dermatogen were selected for measurements. They appeared to be more suitable than others since they showed less variation in different species than the cells of the periblem; this was probably due to their superficial position in the root and corresponding possibility to grow more freely as compared with cells surrounded, and therefore compressed, from all sides by other cells. It should be further expected that the physiological conditions, such as water and oxygen content, would be more uniform in the cells of dermatogen because of their exposed position: Certain morphological peculiarities, moreover, as well as complete lack of intercellular spaces in the dermatogen, facilitated the obtaining of reliable results of measurements; this will be shown in detail later.

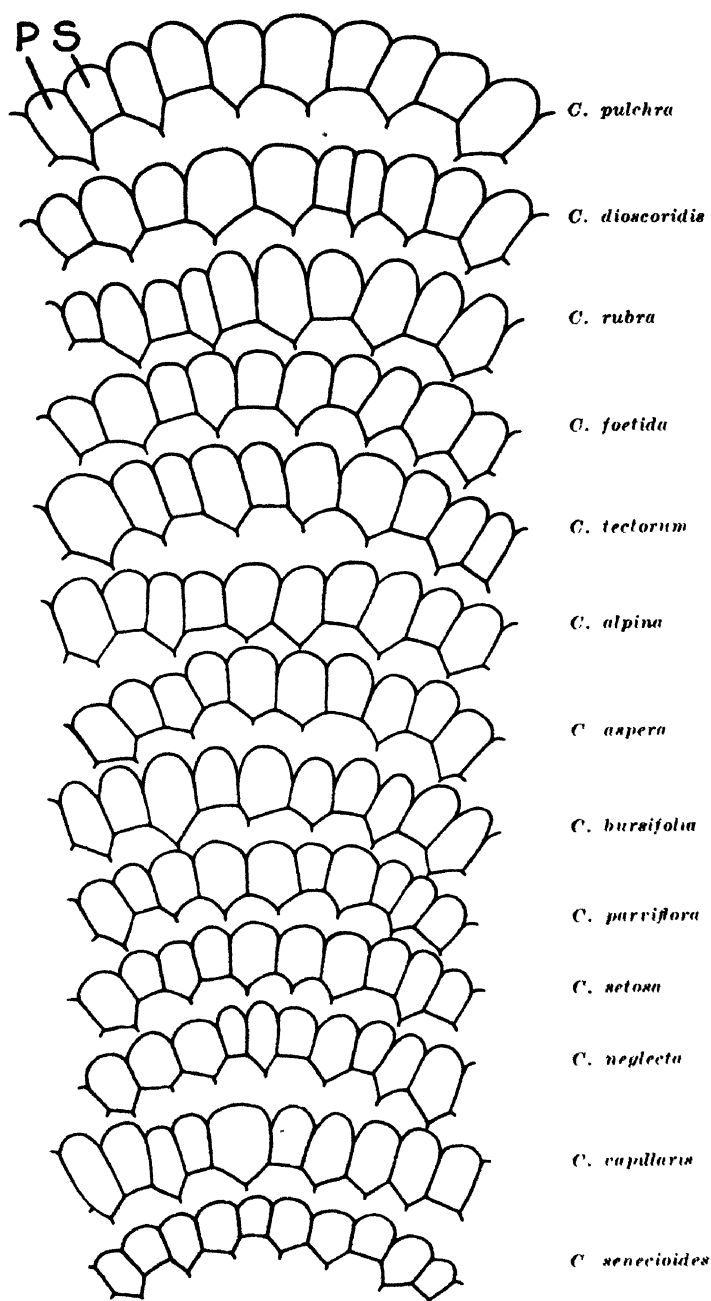


Fig. 1. Portions of the dermatogen of the thirteen *Crepis* species drawn to the same scale. The typical dimorphism of cells expressed in the occurrence of cells of the two kinds (primary cells, P, and secondary cells, S) may be seen, as well as the differences in cell areas of different species.

Finally, it was found, that the sizes of the cells stand in a certain relation to the number of cells present in the whole root, probably owing to differences in mutual pressure and of conditions of nutrition; this source of error was less pronounced in the cells of the dermatogen.

Several tests showed that, owing to the nature of the cell shapes, only direct measurements of the areas on cross-sections could be applied. Measurements of linear, tangential, and radial dimensions, on the contrary, appeared to be impracticable. This can easily be seen from an examination of figure 1, representing the typical cells of the dermatogen of all thirteen species investigated.

The following method of measuring cell areas was devised; adjacent cells along the circumference of the cross-section of the root were drawn to the same scale by means of the camera lucida. The magnification applied was equal to 1400 diameters. A sample consisting of ten adjacent cells was used for obtaining the average area of a cell. Each root gave from four to six samples, according to the number of cells present. The drawn samples of ten cells each were then cut out with scissors and weighed; a set of weights measured directly in square microns was prepared from the same paper as that which was used for the drawings.

This simple, although laborious method of making measurements proved to be far better than any other possible way, including the use of a planimeter. In order to check the accuracy of the measurements, the same sample of cells was drawn and weighed repeatedly; the error of measurements was found to be negligible for it never exceeded 1 per cent of the measured value. Naturally, before using it, the package of paper was tested as to its uniformity; it was found that the weight varied from sheet to sheet to a negligible degree. The uniformity of the paper was tested, nevertheless, repeatedly during the progress of the work.

It was of the greatest importance to determine, as a preliminary step, the amount of variation of cell areas along the length of the root in cross-sections belonging to different levels. Several samples from different levels were measured; it was found that the cell area rapidly increased toward the base of the root.

This region of growth, however, occupied only the short conical part of the root; but, as soon as the root assumed nearly cylindrical shape, the growth ceased immediately and the cell areas remained constant, so that it appeared immaterial from what part of the root the cell samples were taken, provided they did not belong to the conical

apex of the root. (The cylindrical part of the root usually begins at the level where only one cell layer of the root cap is present. All measurements were confined to this part of the root.) Table 1, representing some data of measurements of cells belonging to different levels of the cylindrical part of the root clearly demonstrates this.

TABLE 1

AVERAGE CELL AREAS BELONGING TO DIFFERENT LEVELS OF THE CYLINDRICAL PART OF THE ROOT. THE LEVELS ARE 30 MICRONS APART.

DATA OBTAINED ON *Crepis capillaris*

| Levels     | 1   | 2   | 3   | 4   |
|------------|-----|-----|-----|-----|
| Samples: 1 | 215 | 230 | 215 | 210 |
| 2          | 220 | 225 | 215 | 220 |
| 3          | 225 | 220 | 220 | 235 |
| 4          | 210 | 230 | 225 | 225 |
| 5          | 190 | 165 | 170 | 190 |
| Average    | 212 | 214 | 209 | 216 |

It soon was found, however, that simple average areas obtained from samples could not be used for comparative purposes, owing to the fact that the cells of dermatogen were found to be of two different kinds, the larger primary and the smaller secondary cells (see fig. 1). The two kinds of cells greatly differed in size, the average ratio being equal to 1.5; the presence of secondary cells thus considerably reduced the average figures obtained simply by dividing the total area of a sample by ten ( $\equiv$  the number of cells in a sample). From the figures given in table 2 it is plainly evident that the areas of the two kinds of cells do not even overlap but form a typical bimodal distribution. This cell dimorphism was not peculiar to the dermatogen; on the contrary, it occurred in any part of the root where regular cyclic rows of cells were present. Obviously, it was an inevitable result of growth and cell multiplication.

The cells of the two kinds did not alternate regularly; consequently their relative proportions could vary in different individual samples. Furthermore, different species and occasionally different individuals of the same species had different average proportions of the cells of the two kinds. All these circumstances made it necessary to devise some method of correcting the errors of measurements resulting from intra- and interspecific variation in the proportions of primary and secondary cells. As has been shown above, the typical

TABLE 2

COMPARISON BETWEEN PRIMARY AND SECONDARY CELLS. THE FIGURES REPRESENT THE AVERAGE AREAS OF CELLS OF THE TWO KINDS FOR EACH SAMPLE. DATA ON *Crepis pulchra*

| Areas      | Primary         | Secondary       |
|------------|-----------------|-----------------|
| Samples: 1 | 286             | 167             |
| 2          | 250             | 163             |
| 3          | 243             | 150             |
| 4          | 220             | 150             |
| 5          | 275             | 188             |
| 6          | 267             | 163             |
| 7          | 292             | 200             |
| 8          | 307             | 167             |
| 9          | 300             | 150             |
| 10         | 250             | 175             |
| 11         | 267             | 188             |
| 12         | 242             | 188             |
| 13         | 283             | 150             |
| 14         | 300             | 200             |
| 15         | 295             | 175             |
| 16         | 270             | 170             |
| 17         | 229             | 150             |
| 18         | 320             | 180             |
| 19         | 225             | 163             |
| 20         | 225             | 150             |
| 21         | 260             | 190             |
| 22         | 280             | 190             |
| 23         | 270             | 200             |
| 24         | 290             | 220             |
| 25         | 283             | 200             |
| 26         | 250             | 170             |
| 27         | 292             | 175             |
| 28         | 275             | 200             |
| 29         | 270             | 190             |
| 30         | 267             | 175             |
| 31         | 250             | 163             |
| Mean       | 271.3 $\pm$ 3.0 | 181.3 $\pm$ 2.3 |

ratio of the average areas of primary and secondary cells closely approximated 1.5. The necessary correction, however, could not be obtained by simple arithmetical computation since it had been found that the average sizes of the cells changed at a higher rate than one might expect assuming the two kinds of cells to be independent one from another. Table 3 presents the corresponding data.

Since the computation of the correlation coefficient did not seem to be profitable for determination of the nature of relation between both variables, owing to the low number of classes, a graphical method was employed and a straight line was fitted by the method of least squares.

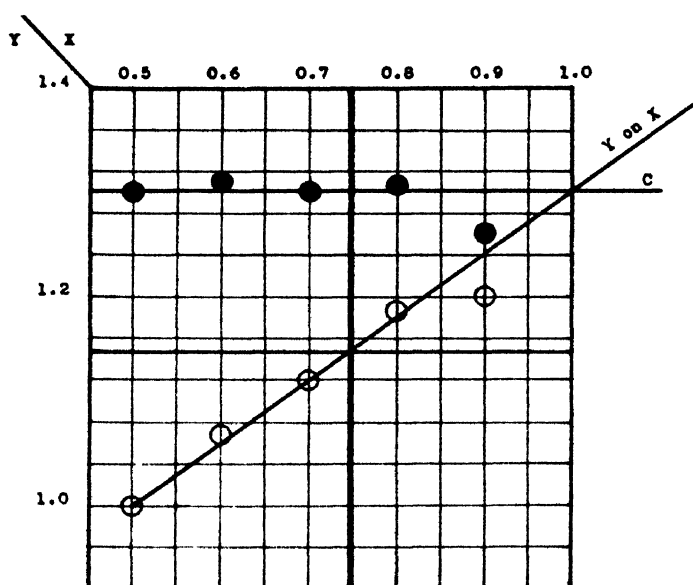


Fig. 2. Graph representing the method of correcting the errors of measurements of cell areas, due to variation in the proportion of primary cells in cell samples.  $X$  = proportion of primary cells;  $Y$  = corresponding relative average area of one cell. Circles represent the observed values, disks, the corrected values. The horizontal line  $C$  represents the theoretical correction level, corresponding to the conditions prior to any cell divisions and, correspondingly, to differentiation of the two kinds of cells.

A nearly linear correlation between the proportion of primary cells and the average area of the cells was found; so that the desired correction could be readily determined. It seemed to be feasible to devise the correction in such a way as to bring the area of the cell up to the value which it would assume in case no secondary cells were to be formed, i.e., if no cell divisions took place. Figure 2 gives a diagrammatic representation of the method employed.

TABLE 3

RELATION BETWEEN THE PROPORTION OF PRIMARY CELLS AND THE AVERAGE AREA OF A CELL. DATA OBTAINED ON 11 DIFFERENT SPECIES, REPRESENTED BY 350 SAMPLES OF 10 CELLS EACH. THE FIGURES REPRESENT RELATIVE VALUES OBTAINED BY TAKING THE AREA CORRESPONDING TO THE PROPORTION 0.5 = 1.0

| Proportion of primary cells | 0.5  | 0.6  | 0.7  | 0.8  | 0.9  | 1.0  |
|-----------------------------|------|------|------|------|------|------|
| Relative areas:             |      |      |      |      |      |      |
| Observed                    | 1.00 | 1.07 | 1.12 | 1.19 | 1.20 |      |
| Expected                    | 1.00 | 1.04 | 1.08 | 1.12 | 1.16 | 1.20 |

TABLE 4

CORRECTION COEFFICIENTS COMPUTED FOR VARIOUS PROPORTIONS OF PRIMARY CELLS

| Proportions of primary cells | 0.5   | 0.6   | 0.7   | 0.8   | 0.9   | 1.0   |
|------------------------------|-------|-------|-------|-------|-------|-------|
| Coefficients                 | 1.299 | 1.226 | 1.160 | 1.102 | 1.048 | 1.000 |

TABLE 5

COMPARISON BETWEEN CORRECTED AND UNCORRECTED AVERAGE CELL AREAS IN DIFFERENT *Crepis* SPECIES

| Average areas                      | Proportions of primary cells |     |     | Coefficients of variation |
|------------------------------------|------------------------------|-----|-----|---------------------------|
|                                    | 0.5                          | 0.6 | 0.7 |                           |
| <i>C. pulchra</i> :                |                              |     |     |                           |
| Corrected                          | 292                          | 293 |     | 3                         |
| Uncorrected                        | 225                          | 240 |     | 6.7                       |
| <i>C. tectorum</i> :               |                              |     |     |                           |
| Corrected                          | 282                          | 289 |     | 2.4                       |
| Uncorrected                        | 217                          | 236 |     | 8.8                       |
| <i>C. neglecta</i> :               |                              |     |     |                           |
| Corrected                          | 194                          | 191 |     | 1.5                       |
| Uncorrected                        | 149                          | 156 |     | 4.7                       |
| <i>C. capillaris</i> :             |                              |     |     |                           |
| Corrected                          |                              | 240 | 240 | 0.0                       |
| Uncorrected                        |                              | 196 | 207 | 5.6                       |
| <i>C. parviflora</i> :             |                              |     |     |                           |
| Corrected                          |                              | 174 | 180 | 3.4                       |
| Uncorrected                        |                              | 134 | 147 | 9.7                       |
| Average coefficients of variation: |                              |     |     |                           |
| Corrected                          |                              |     |     | 1.5                       |
| Uncorrected                        |                              |     |     | 7.1                       |

On the basis of the above data, constant correction coefficients were computed, each one corresponding to a certain proportion of primary cells in one given sample; through simple multiplication by the corresponding coefficient and dividing by ten the average area of a given sample was transformed into a corrected value for one cell. The values thus obtained represented the maximal cell areas which would arise when all modifying influences, including cell divisions, are eliminated. Table 4 gives these coefficients together with corresponding proportions of primary cells.

In order to check the results of the correction applied, uncorrected measurements were compared with the corrected ones in regard to degree of variability. Table 5 gives the corresponding data for several *Crepis* species. From this table it is evident that the use of this correction greatly reduces the variation in measured areas, thus eliminating the influence of variation in the proportions of primary cells.

The whole method of measuring cross-radial areas of cells then assumed the following form. First, samples of ten cells each were drawn, cut, and weighed in terms of square microns; to every figure thus obtained was added a note regarding the corresponding proportion of primary cells. Second, the corresponding coefficient was set in the computing machine and all the figures obtained by direct measurement were corrected through multiplication by this coefficient and division by ten; first for samples containing five primary cells, then for those containing six, and so on.

The corrected areas obtained were treated as individual observations in calculating the mean values and other constants. The number of samples measured was never less than 25; as will be seen later, this number was sufficient to obtain fairly consistent and reliable results. Nevertheless, a much greater number of samples was measured in the majority of cases; altogether over 1100 samples were measured representing more than 11,000 individual cells.

After the true corrected values for cell areas were obtained, it remained to find the length of the cells along the axis of the root in order to determine the actual volume. This apparently simple task, however, proved to be an extremely difficult one, owing to the very high and variable rate of cell division and, correspondingly, to an extreme variation in cell size. In view of this difficulty it was decided simply to calculate the volumes through multiplication of the areas by their square root. This method seemed to be justified, especially when relative values were needed. One might suppose, however, that



the primary cells could be proportionally shorter than the secondary ones, the respective volumes being thus about the same. Although, a priori, such an assumption seemed to be highly improbable, yet it was checked by direct comparison of both kinds of cells on longitudinal sections. It was found that there is no detectable difference in relative length between primary and secondary cells, the two kinds showing the same range of variation. Inequalities in cell length in different species were always clearly pronounced, provided the species in question differed in cell areas. Although there was considerable difference, the results of measurements could not be evaluated because of extreme variation in proportion of cells of different length.

As measures of the chromatin mass, the values given by Mann-Lesley (1925) and expressed in relative chromosome lengths were used, with the exception of *C. senecioides* which was not included in her work.

## THE RESULTS OF MEASUREMENTS OF CELL VOLUMES IN THIRTEEN CREPIS SPECIES

Applying the method described above the following mean values were obtained for cell areas and calculated volumes in the thirteen species investigated (see table 6). From this table it may be seen that the cell areas and correspondingly the calculated cell volumes differ widely in different species. Among the thirteen species one may distinguish groups characterized by similarity of cell size. Thus *C. dioscoridis*, *C. pulchra*, and *C. rubra* all have very similar large cells; *C. foetida*, *C. bursifolia*, *C. capillaris*, and *C. alpina* have medium-sized cells; *C. parviflora*, *C. neglecta*, and *C. setosa* form a group with small cells; *C. senecioides* stands apart with its very small cells.

## RELATION BETWEEN CHROMATIN MASS AND CELL VOLUME

The length of somatic chromosomes as they appear in metaphase, was used as a measure of the chromatin mass. It is obvious that the length of the chromosomes may be applied as a measure of chromatin volume only when the width of the chromosomes is constant. As a matter of fact, however, this is not the case; the different species have in many instances chromosomes of very different widths. The measurement of chromosome width appears now to be extremely difficult owing to its exceedingly low value and especially to variation resulting from

the degree of splitting of each chromosome, the latter being always composed of two daughter strands. It is sufficient at present to understand that chromosome length alone cannot be applied without due reservations.

TABLE 6  
CELL AREAS, CALCULATED CELL VOLUMES, AND RELATIVE TOTAL LENGTH OF CHROMOSOMES IN THIRTEEN SPECIES

| Species               | Cell area in square microns | Calculated volume in cubic microns | Corrected average of total length of chromosomes* |
|-----------------------|-----------------------------|------------------------------------|---|
| <i>C. senecioides</i> | 156 8 $\pm$ 2.8             | 1963 $\pm$ 35                      | 42 0  |
| <i>C. parviflora</i>  | 180 3 $\pm$ 2.2             | 2421 $\pm$ 30                      | 69 9  |
| <i>C. neglecta</i>    | 192 0 $\pm$ 2.9             | 2660 $\pm$ 40                      | 61 7†   |
| <i>C. setosa</i>      | 201 0 $\pm$ 3.0             | 2850 $\pm$ 43                      | 63 2  |
| <i>C. aspera</i>      | 234 6 $\pm$ 2.6             | 3693 $\pm$ 38                      | 82 6  |
| <i>C. foetida</i>     | 248 3 $\pm$ 2.7             | 3913 $\pm$ 43                      | 93 7  |
| <i>C. bursifolia</i>  | 249 0 $\pm$ 3.6             | 3929 $\pm$ 57                      | 78 5  |
| <i>C. capillaris</i>  | 250 9 $\pm$ 5.3             | 3974 $\pm$ 84                      | 61 4†   |
| <i>C. alpina</i>      | 256 7 $\pm$ 7.1             | 4113 $\pm$ 114                     | 87 3  |
| <i>C. tectorum</i>    | 275 5 $\pm$ 2.2             | 4573 $\pm$ 37                      | 88 7  |
| <i>C. dioscoridis</i> | 290 2 $\pm$ 4.0             | 4944 $\pm$ 68                      | 109 4   |
| <i>C. pulchra</i>     | 296 3 $\pm$ 2.4             | 5100 $\pm$ 41                      | 112 1   |
| <i>C. rubra</i>       | 296 8 $\pm$ 3.9             | 5113 $\pm$ 67                      | 102 9   |

\* From Mann (1925) table 3, p. 302 (except *senecioides*).

† These species have thicker chromosomes, cf. p. 223.

TABLE 7  
CELL AREAS AND CALCULATED VOLUMES OF THE MEMBERS OF A POLYPLOID SERIES IN *C. capillaris*

| Chromatin mass                              | Cell areas | Cell volumes   |
|---|------------|----------------|
| Haploid (one plant, several roots)          | 147.7      | 1789 $\pm$ 22  |
| Diploid (several plants, one root of each)  | 250.9      | 3960 $\pm$ 84  |
| Triploid (several plants, one root of each) | 329.0      | 5968 $\pm$ 82  |
| Tetraploid (three plants, one root of each) | 425.4      | 8976 $\pm$ 100 |

Prior to comparing different species in the matter of the relation between their chromatin masses and the respective cell volumes, a study of intraspecific variability of chromatin masses was made with the expectation that these conditions would be clearer, i.e., unobscured by eventual specific developmental peculiarities or errors due to inaccurate chromosome measurements. For this purpose a polyploid series in *C. capillaris* was used, consisting of haploid, diploid, triploid, and tetraploid individuals. The figures obtained from measurements of cells of the representatives of this polyploid series are given in table 7.

Essentially the same relations between chromatin mass and cell volume were found in the polyploid series of the two other species: *C. tectorum* (2n, 3n, and 4n were investigated) and *C. parviflora* (2n and 3n). The calculated cell volume here was also found to be practically proportional to the chromatin mass.

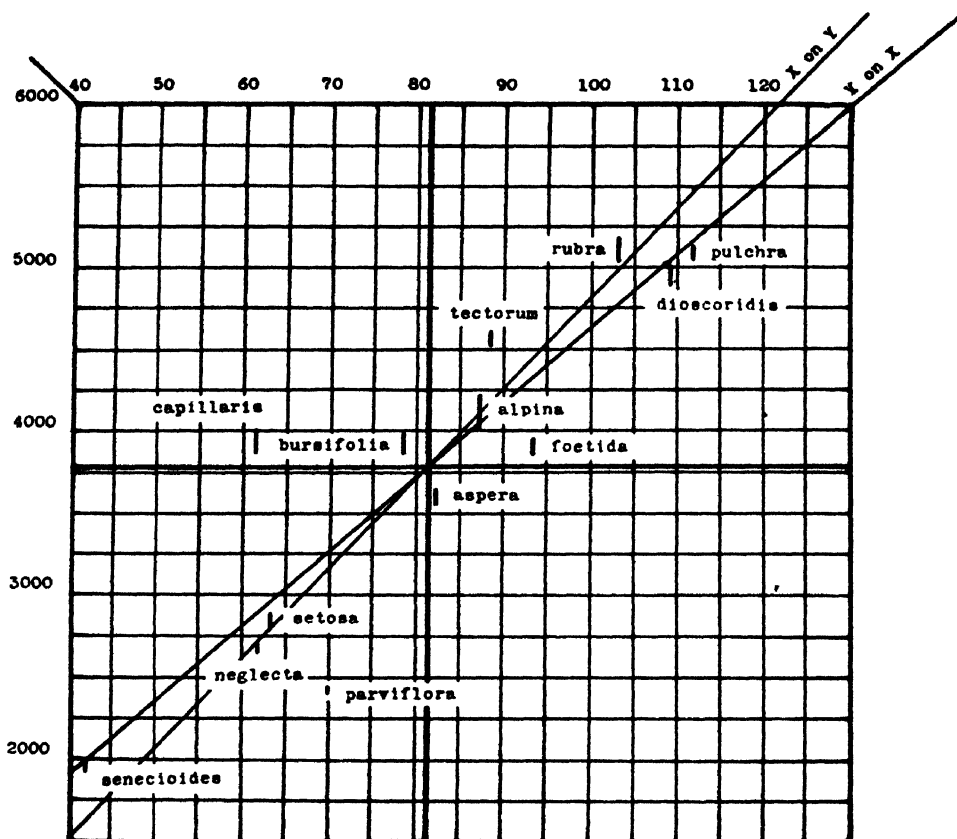


Fig. 3. Graphic representation of the relation between chromatin mass and cell volume in thirteen *Crepis* species. The length of the bars, indicating different species, corresponds to the respective probable error. The cell volume is expressed in cubic microns; the chromatin mass, in relative units of chromosome length.

As was stated above, these data on the polyploid series were secured chiefly in order to check the method employed; the correctness of which, as may be seen, they have established. As for the accurate determination of the degree of correlation between chromatin mass and cell volume in the polyploid series, the actual computing of the correlation coefficient did not seem to be practicable on account of the few classes of variables.

Different species could now be compared safely in the matter of the relation between their chromatin masses to the respective cell volumes.

The usual method of graphic representation was here employed. Figure 3 gives the corresponding correlation diagram for all thirteen species investigated. This diagram clearly shows that there is a very strong positive correlation between the chromatin mass and the cell volume, save for a few exceptions. These exceptional species deviate in the graph from the expected positions to a significant degree; but, as will be shown in the discussion, this circumstance has its natural explanation.

Computation of the coefficient of correlation gave the value,  $r = .90 \pm .04$ . Computation of the correlation ratio did not seem to be practicable owing to the low number of classes; it appears from the graph shown in figure 3, that the correlation may be regarded as linear.

### DISCUSSION AND CONCLUSIONS

That there is direct relation between the sizes of cells and the nuclei they contain, has been well known since the time of Strasburger (1893). Later, on the basis of numerous observations, R. Hertwig (1903, 1908) developed the idea of the "nucleoplasmic ratio." This theory contends that the quotient, nucleus/cytoplasm, is a constant of fundamental importance for the life activity of the cell. Further investigations have shown, however, that the nucleoplasmic ratio varies very much in different tissues (Kleinberger, 1917); it varies under external influences as well, especially those of temperature (R. Hertwig, 1903; O. Hartmann, 1919). The most striking instances of change of the nucleoplasmic ratio is furnished by sexual cells, the male cells containing practically no cytoplasm, the female cells developing a vast amount of cytoplasmic material. From these and many similar data it is evident that the nucleoplasmic ratio could hold true only for cells prior to any differentiation, specialization, or modifying influences of the surrounding medium.

All the above mentioned data were obtained from studies of resting nuclei, their sizes being regarded as a measure of the amount of nuclear material. It may be observed, however, that the resting nuclei may greatly differ in the quantity and quality of materials they contain, in connection with innumerable changes they undergo in different stages of the cell life, cell division, specialization, and differentiation of cells, etc. Thus the data obtained from observation on "resting" nuclei may be regarded as scarcely reliable; at least they should not be accepted without due reservations.

The comparison of nuclei on the basis of their actual chromatin content, which can be measured in a definite stage of development, gives much more conclusive results. The actual counts and especially the measurement of chromosomes at the metaphase give directly the relative amount of chromatin, as it emerges at this critical moment of karyokinesis.

The first to demonstrate the effect of change of chromatin amount was Gerassimoff (1902), who experimentally produced a diploid gametophyte of *Spirogyra*. Gerassimoff found that the diploid cells greatly exceed in size the normal haploid cells. Thus it was demonstrated that an increase in the actual amount of nuclear substance stands in causal relation to the cell size and, consequently, to the general amount of protoplasm.

Gerassimoff's results were immediately confirmed by Boveri (1905) on animal material. From his famous experiments on *Echinodermata* eggs this investigator concluded that even the addition of a single supernumerary chromosome causes an enlargement of the cell. From his measurements he found that not the volume but the surface of the cell is proportional to the chromatin mass it contains.

Following Gerassimoff and Boveri many investigators became interested in this problem. Tischler on *Musa* (1910), the Marchals on mosses (1906, 1907, 1909, 1911), Winkler on *Solanum* (1916), and especially Gates on *Oenothera* (1909) have found that an increase in chromatin material causes also an increase in the masses of nuclei and of the cytoplasm. The figures obtained from measurements by some of these investigators showed, however, that there was no straight proportionality between chromatin mass and cell size. The cell size was, furthermore, strikingly variable in different tissues of the same organism; this is, of course, to be expected as a natural result of differentiation. It seems strange, therefore, that none of these workers measured the cells of the primary meristem of the root. Nemec (1910), who was the first to find tetraploid cells in normal tissues, worked with root tips; he did not make any measurements.

To cite more recent data, Taylor's work on *Acer* (1920), Blakeslee's on *Datura* (1922), and especially F. Wettstein's investigations on experimental polyploidy in mosses (1924) should be mentioned. Most recently Clausen and Goodspeed (1925) have shown that even under hybrid conditions the cell size tends to correspond to the chromatin mass.

All these and many other similar data proved beyond question that there is a definite relation between chromatin mass and cell size. In the great majority of these cases the differences in chromatin mass were due to polyploidy, i.e., to the addition of entire chromosome sets.

As a matter of fact, however, natural species show differences in chromatin mass owing to variation in chromosome size, the chromosome number being often the same. At present, data concerning the relation between cell size and chromatin mass as determined by the sizes of the chromosomes, are entirely lacking, save for a few observations which contradict one another. Such specific differences as inequalities in chromosome size seem to be more important than variations resulting from polyploidy, since the latter could produce only secondary new forms from pre-existing species which had arisen by different processes. In other words, a new species could not arise through polyploidy, unless sufficient specific changes in the organization of the chromosomes themselves should take place. The visible results of these phylogenetic changes probably represent alterations in both the relative and absolute sizes of chromosomes (M. Navashin, 1925, 1926; L. Delaunay, 1926).

Tischler was probably the first worker who became interested in this problem. Comparing a giant strain of *Phragmites* ("var. *Pseudodonar*") with a normal one (1918), he found it to possess much larger meiotic chromosomes, but the size of pollen mother cells was unaffected by this difference in chromatin content.

Delaunay reached essentially the same conclusion, while measuring meristematic cells (dermatogen of the root tips) in two *Muscari* species (1926). This investigator deduced from his measurements that the cell volumes in the two species investigated were identical, in spite of significant differences in chromosome sizes. He found the number of meristematic cells, not their sizes, to be connected with the chromatin mass.

Most recently Jaretsky (1928), studying various *Polygonaceae*, came to an opposite conclusion; he has found that there is direct connection between chromosome size and cell size. But he offers no measurements in proof of his statement.

It does not seem difficult to explain the contradictory results obtained by the above mentioned investigators. First, it may be noted that Tischler might have dealt merely with that type of size difference which was not due to actual variation in chromatin mass, but was

rather connected with physiological conditions, such as those known to occur in different tissues of the same organism. The figures presented by Tischler do not give a clear idea as to the nature of chromosome differences in the two *Phragmites* strains studied by him.

In Delaunay's case there is not the slightest doubt that the two species investigated (*Muscari longipes* and *M. tenuiflorum*) actually differ in chromatin amount, the respective total chromosome length being in the ratio 1.35:1.00. From the data of this writer, one may easily see, however, that his cell measurements could have been subject to errors, owing to the same general causes as those described in detail in the present paper. Thus Delaunay merely applied average values of the linear dimensions of cells; he did not select cells, but probably used random samples of cells (90 cells of each species) for determination of the radial and the tangential linear dimensions. The axial length of cells was determined simply by dividing the total length of the meristematic zone by the number of cells present in a longitudinal section. Referring now to what was found by the present writer, it may easily be seen that, if Delaunay's two species differed in the proportion of primary cells, his conclusions regarding the radial and tangential dimensions could have no significance; not to mention the impossibility of obtaining a reliable linear measure of such a complex shape. To illustrate this, suppose that *M. longipes* has 0.5 of primary cells and *M. tenuiflorum*, 0.7. If the relations between the two kinds of cells are similar to those found in *Crepis* (which is highly probable, since in several of the Liliaceae the present writer found exactly the same conditions as in *Crepis*), it would, of course, mean that the average areas of cells obtained from random sampling might happen to be equal, the real mean values being in the ratio 1.12:1.00, which would give the ratio for volumes approximating 1.2:1.0; and this latter ratio is not so far from the ratio of chromatin masses in the two species. Some details mentioned by Delaunay directly suggest the probability of the existence of a difference in the proportions of primary cells in the two species, although he does not even mention the occurrence of cells of the two kinds. Thus, Delaunay has found that *M. longipes* has narrower but longer cells as contrasted with *M. tenuiflorum*. It seems to be very probable, therefore, that the former species has actually fewer primary cells, since the width of cells is above all affected by the proportion of additional (secondary) cells. As for the axial dimensions of cells, there is no simple way to eliminate the extremely great error arising from variation in the rate

of cell division. In Delaunay's case it would be expected that *M. longipes*, having more cells would be apt to have shorter cells, owing to the fact that the larger number of cells is merely the result of a higher rate of cell divisions. Consequently Delaunay's divergent conclusions can by no means be regarded as established. With regard to his conclusion concerning the connection between chromosome length and the number of meristematic cells, the present writer can not confirm them on his material. Although in some cases a parallelism between chromosome length and the number of meristematic cells could be observed, in other cases both characteristics seemed to vary independently. For example, *C. pulchra* has a very short meristematic zone and very long chromosomes, while *C. tectorum* possesses much shorter chromosomes but about twice as many meristematic cells.

The data of the present investigation, however, indicate that there is a strict proportionality between chromosome length and cell volume in related species; this proportionality is not less in this instance than in the polyploid series. Some deviations from the expected relations, however, seem to contradict the generality of this conclusion. Thus *C. capillaris* appears to have larger cells than might be expected from the chromosome measurements; however, *C. neglecta* with chromosomes almost as long as those of *C. capillaris*, possesses much smaller cells. But this apparent disagreement has its simple explanation. As has been stated, in comparing the chromatin masses in different species only the total length of the chromosomes was considered, without reference to chromosome width. It is therefore apparent that in case the chromosomes differ in width, the length no longer can be used as a relative measure of volume. This is precisely the situation in *C. capillaris* and *C. neglecta*, as a glance at the chromosomes of the two species is sufficient to show, the former having considerably thicker chromosomes than the latter (cf. Hollingshead and Babcock, 1930, fig. 11, p. 19). The same difference exists also in several other species.

These differences in relative chromosome length and width raise a very important question as to the relation between the general chromosome shape and the actual amount of the chromatin content. As may easily be seen, the shape of the colloidal body of a chromosome is determined to a large extent by the properties of the surrounding medium, especially by its acidity, osmotic pressure, etc. Very considerable changes in the shape may, therefore, occur when the conditions of the cytoplasm become changed. This was actually demonstrated by



Kuwada and Sakamura (1926), who succeeded in changing the size of the chromosomes by varying the hydrogen ion concentration. It is natural to suppose, however, that different species may differ in the acidity of their protoplasts, so that in some of them the chromosomes may be more contracted (owing to higher acidity), others, on the contrary, may be more elongated (owing to lower acidity). These alterations may go even farther than simple changes of shape due to variation in surface tension; for the amount of water contained in the chromosome body may vary considerably, thus changing the actual mass of the chromosome itself. That the cause of variation in relative width and length of the chromosomes is not inherent in the chromosomes, may be deduced from the fact that chromosomes belonging to the same plate never show differences in widths. This very plausible conclusion leads Jaretsky (*loc. cit.*) to explain certain variations in chromosome shape through differences in the acidity of the cytoplasm.

It is now perfectly clear that a comparison of chromosome masses of two different species cannot safely be made unless the chromosomes are observed under precisely identical conditions of surrounding medium. Such conditions can exist only in one case: viz., when the chromosomes of the two species are located in the same cell. There is an easy way to accomplish this—by bringing the specific chromosomes together by means of hybridization. The present writer has made a large number of comparative observations on  $F_1$  interspecific hybrids (1929), and it was found that the parental chromosomes never differ in width in hybrids, even though they previously displayed marked differences in the pure species. It could further be observed that this equality in width was accompanied by corresponding changes in chromosome length; thus, for instance, in a hybrid, *C. capillaris*  $\times$  *C. neglecta*, the *neglecta* chromosomes appeared as thick as those of *capillaris* but this widening of the chromosomes was accompanied by considerable shortening. Table 8 gives the results of chromosome measurements of some pure *Crepis* species and of hybrids between them.

TABLE 8

THE TOTAL LENGTH OF HAPLOID CHROMOSOME COMPLEXES MEASURED IN CELLS OF PURE SPECIES AS COMPARED WITH THAT OBTAINED FROM MEASUREMENTS IN CELLS OF CORRESPONDING INTERSPECIFIC HYBRIDS. ROOT TIPS

| Total haploid chromosome length of | From pure species | From <i>capillaris</i> $\times$ <i>neglecta</i> hybrid | From <i>capillaris</i> $\times$ <i>lectorum</i> hybrid |
|------------------------------------|-------------------|--|--|
| <i>C. capillaris</i> . . . . .     | 107.94 $\pm$ .40  | 125.85 $\pm$ .69                                       | 109.43 $\pm$ .47                                       |
| <i>C. lectorum</i> .               | 155.55 $\pm$ .88  | .....  | 147.57 $\pm$ .50                                       |
| <i>C. neglecta</i> .               | 97.02 $\pm$ .51   | 83.33 $\pm$ .37  | .....  |

It appears, therefore, that the length-width ratio of a chromosome is merely a matter of the chemico-physical condition of the cytoplasm; and the situation would not change at all, if one assumes that the properties of the chromosome itself are responsible (to a certain extent at least) for these conditions. Further discussion of this problem will appear elsewhere.

It is clear that comparative measurements of chromosome volume are relatively easy when hybrids are used. It may give reliable results also as far as comparison of pairs of species is concerned. But the conclusions may not be applied without reservations to a larger number of species, since it is obvious, that the conditions in a hybrid cell are determined by the cooperation of the two specific chromosome sets. And, if in certain cases actual change of the absolute chromosome mass should take place, the same species might appear to have different chromosome volumes, depending upon the other species with which it had been crossed. Nevertheless, judging from the available data, it does not seem to be probable that the chromosome changes often go beyond mere shortening and proportional thickening; the total volume being unaffected.

Through comparative study of hybrid chromosomes, it was found that *C. capillaris* has actually much more bulky chromosomes than *C. parviflora*, *C. neglecta*, and even *C. setosa*. Therefore the respective positions of these species in the diagram of figure 3 should be correspondingly changed, thus bringing them much closer to the actual regression line.

As has been shown above, the connection between chromatin mass and cell volume is not less pronounced in interspecific variation, than in intraspecific changes due to polyploidy. This justifies the assumption, with a fair degree of probability, that the same conditions which make the polyploid cells larger, are acting in the case of specific alteration of chromatin mass. Although there is no doubt that members of polyploid series actually differ in the amount of genes present, it might be premature at present to conclude that just this difference in the amount of homologous genes is alone responsible for the variation in the production of protoplasmic material; yet such a conclusion is certainly indicated. In regard to specific differences in chromatin amount, such a conclusion must be made with still more reservations, in spite of its plausibility.

From his studies on *Polygonaceae* Jaretzky (*loc. cit.*) has made much more categorical assertions, namely, that the species having

larger chromosomes must be regarded as polyploids, the large chromosomes being in reality compound structures, each composed of two or even more small chromosomes. This idea is not new, nor is it warranted by a sufficient number of facts; furthermore, this author reverts to the old idea of loss mutations in order to explain the differences between various species. It is, however, perfectly obvious that regarding loss mutations as a cause of evolution, one does not avoid the absurd consequences of such an idea by appealing to polyploidy, irrespective of its kind.

It may be of interest to discuss briefly another problem arising from the results of the present investigation. From the accurate determination of relative proportions of primary and secondary cells in the dermatogen of the root, it was found that these proportions are remarkably constant for a given root; it appeared further to be very constant for a given species. Table 9 gives some corresponding data.

TABLE 9

PROPORTIONS OF PRIMARY CELLS IN THE DERMATOGEN IN DIFFERENT *Crepis* SPECIES

| Species               | Proportion of primary cells |
|-----------------------|-----------------------------|
| <i>C. parviflora</i>  | 563 $\pm$ .012              |
| <i>C. foetida</i>     | .568 $\pm$ .010             |
| <i>C. senecioides</i> | .567 $\pm$ .014             |
| <i>C. bursifolia</i>  | 570 $\pm$ .011              |
| <i>C. tectorum</i>    | 575 $\pm$ .010              |
| <i>C. pulchra</i>     | 578 $\pm$ .008              |
| <i>C. aspera</i>      | .572 $\pm$ .009             |
|                       | mean value .571 $\pm$ .001  |
| <i>C. capillaris</i>  | .665 $\pm$ .014             |

Besides the proportions given in the preceding table, some others were determined, for example, the occurrence of proportions 0.62, 0.71, and 0.75 (possibly also 0.60) was established, also that in two species the proportion of primary cells sometimes dropped below 0.5; and the most common proportion closely approximated 0.571. Different roots of the same species eventually exhibited different proportions; but never did a proportion occur which was intermediate between those enumerated above. The variation in the proportions of primary cells thus proved to be discontinuous.

It is easy to observe, that, since the proportion of primary cells is merely a direct result of the proportion of preceding cell divisions, the

latter must be strictly limited by a definite number, typically constant for each case. The relations between the proportion of dividing cells and the proportion of primary cells in the resulting tissue are shown in table 10.

TABLE 10  
THEORETICAL RELATION BETWEEN THE NUMBER OF CELL DIVISIONS AND THE  
RESULTING PROPORTIONS OF PRIMARY CELLS

| Relative numbers of<br>dividing cells | Relative numbers<br>of resulting cells of the<br>two kinds | Proportion of<br>primary cells |
|---------------------------------------|--|--------------------------------|
| No divisions                          |  | 1 000                          |
| 1 out of 10                           | 10 prim. + 1 secon.  | 0 909                          |
| 1 out of 5                            | 5 prim. + 1 secon.   | 0 833                          |
| 1 out of 4                            | 4 prim. + 1 secon.   | 0 800                          |
| 1 out of 3                            | 3 prim. + 1 secon.   | 0 750                          |
| 2 out of 5                            | 5 prim. + 2 secon.   | 0 714                          |
| 1 out of 2                            | 2 prim. + 1 secon.   | 0 667                          |
| 3 out of 5                            | 5 prim. + 3 secon.   | 0 625                          |
| 2 out of 3                            | 3 prim. + 2 secon.   | 0 600                          |
| 3 out of 4                            | 4 prim. + 3 secon.   | 0 571                          |
| All dividing                          |  | 0 500                          |

The whole process of differentiation of the two kinds of cells (see fig. 1) may be explained in the following way: after the last cell layer of the meristem is formed through divisions in the radial direction, a series of cell divisions in a perpendicular (tangential) direction takes place; each dividing cell forms two cells, one of which is identical with the original (primary) cell, the other being different in shape and size owing to its position opposite the cell wall of the underlying cell of the row underneath. Thus two kinds of cells arise, the primary, equal in number to the cells of the layer below and characterized by pointed inner ends, which occupy the spaces between the cells of that layer; and the secondary, which are located between the primary cells and opposite the cells of the layer below.

The above table gives only a few of the most simple numerical relations which would arise as the result of various proportions of dividing cells; an indefinitely large number of different combinations might be imagined. As a matter of fact, however, only very few of the possible combinations occur in reality; namely, only the following relative numbers of dividing cells occur: 1 out of 3, 2 out of 5, 1 out of 2, 3 out of 5, 2 out of 3, and 3 out of 4, the latter proportion being the most common (it results in the proportion of primary cells being 0.571).

It seems evident, therefore, that the rate of cell division follows an extremely simple rule: only the simplest numerical ratios between dividing and non-dividing cells are realized. Further discussion of this matter not being pertinent to this paper, it is merely stated that the described relations are undoubtedly consequences of some most fundamental physical property of the cell. The study of these relations might possibly lead to some understanding of the most direct causes of cell division and cell differentiation. Further investigation is in progress.

### SUMMARY

Many closely related species are known to differ greatly in their chromatin content. There is no strict parallelism between the specific chromatin amount and external morphology. This phenomenon remains as yet unexplained.

In view of the fact that in adult organisms the original relation between chromatin mass and the mode of development could be obscured by some influences of certain genes, it was decided to compare the meristematic cells of different species. The size of the cells of the dermatogen of the root was chosen as material for the investigation, on the supposition that the amount of protoplasmic material produced reflects the most fundamental property of the cell, viz., its ability to synthesize a definite amount of organic matter.

A special method of measuring cell volumes was devised; and all sources of error were eliminated, as far as possible.

It was found for the thirteen species of *Crepis* investigated, that the volume of the cell was proportional to the amount of its chromatin. Thus it was established that the specific alterations in chromatin amount have the same primary consequences as the intraspecific variation in the number of the homologous chromosome sets arising through polyploidy. This close analogy suggests the possibility that the specific differences in chromatin amount are connected with the amount of homologous genes, as in the polyploid series.

Some data of the present investigation clearly show that the proportions of cell divisions in primary meristem are strictly confined to a series of simple fractions; viz.,  $\frac{1}{3}$ ,  $\frac{2}{5}$ ,  $\frac{1}{2}$ ,  $\frac{3}{5}$ ,  $\frac{2}{3}$ , and  $\frac{3}{4}$ . This indicates that the cell divisions and, consequently, the resulting simplest cell patterns are controlled by some elementary physical process.

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**BY**

**CHARLES F. POOLE**

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# THE INTERSPECIFIC HYBRID, *CREPIS RUBRA* X *C. FOETIDA*, AND SOME OF ITS DERIVATIVES.

## II. TWO SELFED GENERATIONS FROM AN AMPHIDIPLOID HYBRID

BY

CHARLES F. POOLE

A plant of  $F_1$  *Crepis rubra* x *C. foetida* was isolated from other *Crepis* species in 1927. At maturity the achenes were gathered, and in the following year these gave rise to nine  $F_2$  plants, among which were found 3 diploids, 5 triploids, and 1 amphidiploid. The latter proved to be the first *Crepis* hybrid of this type to exhibit any degree of fertility, possessing 2.7 per cent of the estimated potential number of achenes. Demonstration that new species may arise in *Crepis* through amphidiploidy would prove of great interest, since all but two of the American *Crepis* species occur in chromosome numbers with a base number of 11, a situation which is presumably due to amphidiploidy. Other chromosome numbers in *Crepis* have been assumed to arise through amphidiploidy (Babcock & Navashin, 1930), and the discovery of an amphidiploid with such low fertility was therefore disappointing, although grounds existed for believing it could give rise to fertile and stable recombination products.

From among the rapidly increasing number of amphidiploids in the literature, several indicate sterility of the same order of magnitude as that found in *Crepis rubra-foetida*. One of them, *Nicotiana rustica-paniculata* (Lammerts 1931), has produced four distinct derivative lines, which may indicate similar potentialities in some, if not all, partly sterile amphidiploids. Discovery of stable derivative lines in a *Crepis* hybrid of this type would present certain advantages not to be expected in any amphidiploid thus far known. First, the chromosome numbers of the two parents are considerably lower than in any other known amphidiploid and, second, the somatic chromosome

garniture shows five different morphological types, of which three kinds indicate parental origin.

Furthermore, it was hoped that study of the progeny of this hybrid might throw some light on certain limits encompassing gametogenesis and fertilization. A study of meiosis in this hybrid showed striking irregularities in the pollen mother cells (Poole 1931) at diaphase, first and second metaphase, and the anaphases. The differences clearly showed that it was impossible to distinguish meiotic units as to their valences, since the number of units observed varied from the minimum expected number of quadrivalents, five, to as high as thirteen. It was impossible to say which were univalents of large homologues, quadrivalents of small ones, or any other possible valences, since the range in number indicated the probable presence of all types. It is therefore pointless to attempt examination of meiotic stages in derivative plants until some fairly stable individuals are found. Progress thus far has indicated that derivatives with strikingly different somatic chromosome garnitures, when used as parents will assort at random, producing offspring in the next generation with a wide range in somatic garnitures.

In the following investigation plants of two selfed generations from the  $F_2$  amphidiploid hybrid are studied. Cytological examination was confined to somatic cells in root tips fixed in Navashin's solution and stained with Haidenhein's haemotoxylin. The chromosome garnitures of metaphase plates were drawn with a Spencer camera lucida, at a magnification of 3700. The following drawings are reproduced without reduction. The number of cells studied for each plant varied with the nature of the chromosome rearrangements and the spacing of the chromosomes. *Crepis* species are excellently adapted for such studies, since any root tips that are properly fixed and stained readily reveal any number of reliable plates. Furthermore, advantage was taken of M. Navashin's method of imbedding a half-dozen root tips in a single block.

THE  $F_1$  GENERATION

The first selfed generation,  $F_1$ , of the amphidiploid plant comprised 45 plants, of which 44 reached maturity, and permitted collection of some interesting data. In regard to chromosome number these 45 plants grouped themselves as follows:

| $4n-1$ (19) | $4n$ (20)                 | $4n+1$ (21) | $4n+2$ (22) |
|-------------|---------------------------|-------------|-------------|
| 11          | 23                        | 10          | 1           |
|             | 10 with no rearrangements |             |             |
|             | 13 with rearrangements    |             |             |

Roughly speaking one-fourth of the plants lack one chromosome, and in these plants the absent chromosome may be any one of the twenty comprising the amphidiploid garniture; or any two chromosomes may be lacking, but with the substitution of a third. One-half of the

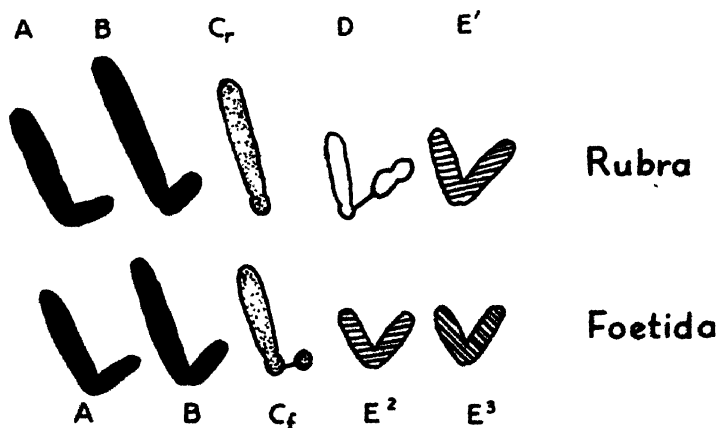


Fig. 1. Illustrating a haploid genom from each parent and showing the five different kinds of chromosomes in the amphidiploid garniture.

population had the full tetraploid number, and of these half appeared to retain the same amphidiploid garniture as the parent, while the remaining half exhibited various rearrangements, involving substitutions of one, two, or three homologues. Finally one-fourth possessed one chromosome extra (in one case two extra); but the extra number was seldom due to the simple addition of a chromosome; rather the additions involved substitutions often as complex as in either of the preceding classes.

Considering the amphidiploid genom as a unit, we may recognize five distinct types of chromosomes, as in figure 1.

The distinction between A and B is not a convenient one to follow, and the four members of each will be referred to as 8A. Two E's are contributed by *rubra*, and four by *foetida*. It is assumed from previous studies that two of these E's from *foetida* conjugate with the highly individual pair of D's from *rubra*, but it is impossible to decide from the total number of six E's which two are concerned. The C chromosomes, however, are clearly differentiated, the two from *rubra* being longer and without satellites, whereas the two from *foetida* are shorter and possess good sized spherical satellites. The C's are never confused with the D's, because the body of the latter is one-half the length of the body of the former and the satellites are not only many times the size of the C satellites, but always appear to have a double nature. This readily recognized amphidiploid set may then be described as 8A 2C<sub>r</sub> 2C<sub>f</sub> 2D and 6E, and for convenience is simply designated 4*n*. In the subsequent tabulations of data from F<sub>3</sub> and F<sub>4</sub> the chromosome garnitures will be indicated as 4*n* plus or minus the appropriate chromosomes.

A fairly complete tabulation of F<sub>3</sub> is given as table 1, in which only two morphological characters are included, since the other dozen characters used in the study lacked correlation with any useful data.

*Chromosome garnitures.*—The 45 plants comprising the F<sub>3</sub> population are arranged in table 1 according to (a) chromosome numbers and (b) the complexity and relationship of their rearrangements. The somatic garniture is represented by a descriptive formula in the right hand column.

An inspection of table 1 discloses a number of valuable facts as to (a) the fertility of certain somatic garnitures and (b) the relation of the two character pairs, nodding-erect buds before anthesis and purple-yellow anther tubes, to the cytological evidence of the presence of the chromosomes in which their genes are believed to have their loci.

In regard to fertility a great disparity exists between plants in the euploid and the aneuploid groups. Nearly all the plants in the euploid group exhibit some degree of fertility, whereas all the plants in the aneuploid group are sterile or practically so, with two exceptions. The relative magnitude of the percentages of good pollen grains in the two groups is equally marked. The correlation between pollen grains and fertility has been treated in a former paper (Poole 1932) but in that paper the complete figures for F<sub>3</sub> were not presented as they are here in table 1. Furthermore, among the euploid plants the



TABLE 1  
GENETIC AND CYTOLOGICAL DATA OF  $F_2$   
20 CHROMOSOME PLANTS

| Plant No. | Bud position | Anther tubes | Per cent good pollen | Per cent fertility | No. of achenes | Formulae              |
|-----------|--------------|--------------|----------------------|--------------------|----------------|-----------------------|
| B 3       | nodding      | tipped       | 22.7                 | 0.35               | 10             | 4n                    |
| 8         | erect        | tipped       | 33.7                 | 0.30               | 7              |                       |
| 9         | nodding      | tipped       | 28.0                 | 3.45               | 90             |                       |
| 12        | nodding      | tipped       | 37.0                 | fertile            | ?              |                       |
| 16        | nodding      | tipped       | 19.4                 | 0.35               | 12             |                       |
| C 3       | nodding      | tipped       | 22.2                 | 0.59               | 17             |                       |
| 7         | nodding      | tipped       | 35.3                 | 2.22               | 66             |                       |
| 10        | nodding      | tipped       | 31.2                 | 0                  | 0              |                       |
| 14        | nodding      | tipped       | 22.4                 | 1.26               | 45             |                       |
| 17        | nodding      | tipped       | 33.9                 | 2.73               | 59             |                       |
| A 1       | nodding      | tipped       | 33.3                 | 1.85               | 30             | - D + E               |
| B 7       | nodding      | tipped       | 56.2                 | 6.71               | 109            |                       |
| C 15      | erect        | tipped       | 47.0                 | 1.93               | 45             | - $C_r + C_t$         |
| 22        | nodding      | tipped       | 42.1                 | 0.07               | 2              |                       |
| B 13      | nodding      | tipped       | 34.4                 | 4.08               | 97             | - $C_t + C_r$         |
| 17        | erect        | tipped       | 31.5                 | 0.56               | 7              |                       |
| C 13      | nodding      | tipped       | 38.2                 | 0.91               | 22             | - $2C_t + 2C_r$       |
| 20        | nodding      | purple       | 19.2                 | 0.77               | 15             |                       |
| C 9       | nodding      | tipped       | 17.3                 | 0                  | 0              | - $C_t + C_r - D + A$ |
| B 18      | nodding      | tipped       | 42.4                 | 8.56               | 158            | - $C_t + C_r - D + E$ |
| C 12      | nodding      | tipped       | 29.5                 | 0                  | 0              | - $C_r - D + E$       |
| 16        | nodding      | tipped       | 39.5                 | 0.10               | 3              | - $C_r + C_t - E + D$ |
| B 6       | nodding      | purple       | 17.4                 | 0                  | 0              |                       |

## 19 CHROMOSOME PLANTS

|      |         |        |       |       |       |                     |
|------|---------|--------|-------|-------|-------|---------------------|
| B 1  | nodding | tipped | 31.6  | 0     | 0     | - $C_r + C_t - D$   |
| C 11 | nodding | yellow | 17.7  | 0     | 0     | - $2C_r + 2C_t - D$ |
| B 19 | nodding | tipped | 27.2  | 2.13  | 59    | - $2C_r + C_t$      |
| C 19 | nodding | yellow | 17.6  | 0     | 0     |                     |
| 2    | .....   | .....  | ..... | ..... | ..... | - $C_r + C_t - A$   |
| B 20 | erect   | tipped | 23.0  | 0     | 0     | - $C_t + C_r - D$   |
| 15   | nodding | tipped | 15.8  | 0     | 0     | - $C_r - D + E$     |
| 22   | nodding | tipped | 30.8  | 0.31  | 12    |                     |
| 2    | erect   | tipped | 12.1  | 0     | 0     | - D                 |
| 10   | nodding | tipped | 23.9  | 0     | 0     |                     |
| 14   | erect   | tipped | 40.7  | 0.01  | 2     |                     |

## 21 CHROMOSOME PLANTS

|      |         |        |      |      |    |                   |
|------|---------|--------|------|------|----|-------------------|
| B 4  | nodding | tipped | 20.4 | 0    | 0  | - $2D + 2E + C_t$ |
| 5    | erect   | tipped | 13.7 | 0    | 0  | - $2D + 2E + A$   |
| C 8  | nodding | tipped | 16.1 | 0.35 | 9  |                   |
| 21   | nodding | tipped | 28.4 | 0.04 | 1  | - $D + 2E$        |
| 23   | nodding | tipped | 25.6 | 0.43 | 18 | + D               |
| C 1  | nodding | tipped | 20.2 | 0    | 0  | + A               |
| 5    | nodding | tipped | 14.9 | 0    | 0  |                   |
| 6    | nodding | tipped | 22.2 | 0.50 | 11 |                   |
| 18   | nodding | tipped | 26.7 | 0.28 | 4  |                   |
| B 11 | nodding | tipped | 21.4 | 1.27 | 56 |                   |

## 22 CHROMOSOME PLANTS

|      |         |        |      |   |   |         |
|------|---------|--------|------|---|---|---------|
| B 21 | nodding | tipped | 26.3 | 0 | 0 | + A + E |
|------|---------|--------|------|---|---|---------|

seven sterile or practically sterile plants are mainly confined to that group at the bottom of the 20-chromosome group in which rearrangements involve more than one pair of homologous chromosomes. Two plants of this group C 9 and B 6, possess one chromosome each unknown to either parent species. C 9 had a chromosome resembling a *rubra*-D with either a duplication or a translocation attached, and B 6 had two normal D's and a third chromosome looking like a normal D minus the large double satellite. The garnitures of both plants are illustrated in figures 2a and 2b.

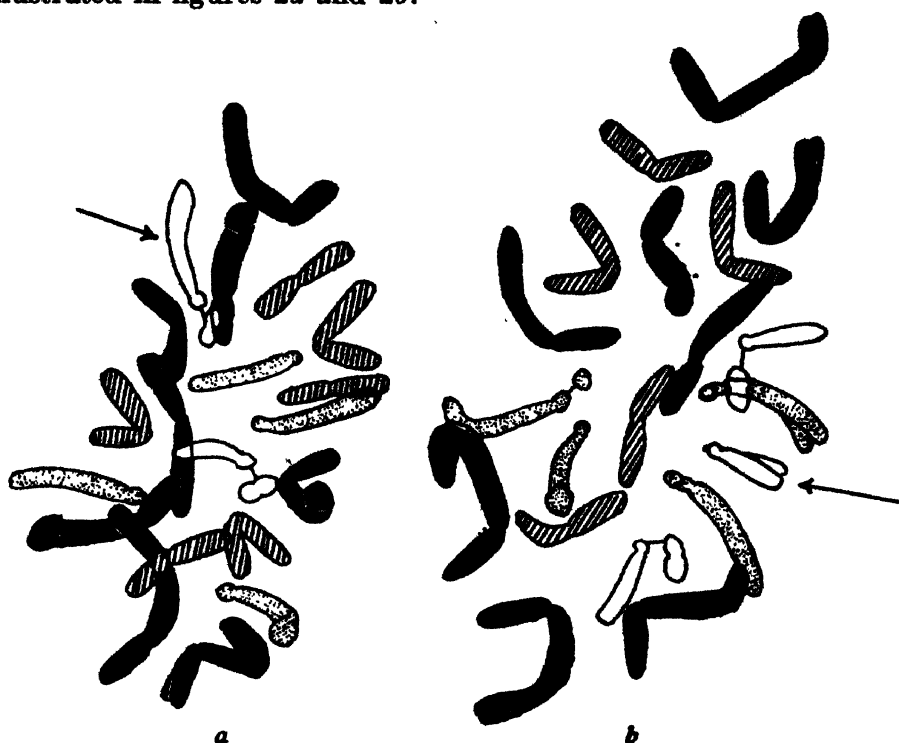


Fig. 2. Two garnitures of  $F_1$  plants each showing new chromosomes as a result of alterations in the D chromosomes: (a) C 9 with a garniture of  $4n-2C_r+2C_r$ , and one of the D's possesses an attachment; (b) B 6 with a garniture of  $4n-C_r+C_r-E+D$ , and one D lacks its satellite.

The most fertile group of plants is that from A 1 to C 20, in which chromosomal rearrangements involve a single pair of homologues. This situation raises the hope that further improvement in fertility lies in the progeny of such plants. One exception exists to the observation that rearrangement in one pair of homologues enhances fertility beyond the amphidiploid set, and that is B 18, the most fertile of the population. The exceptional nature of this plant will be discussed later, because it is not only exceptional among the  $F_1$  population, but also the  $F_2$ , one-half the plants of which were derived from it.

Some significance may be attached to the fact that aneuploid plants are found in the progeny of an amphidiploid. No aneuploid plants have ever been found among derivatives of the cross *C. rubra* x *C. foetida* having a diploid number of chromosomes, whereas aneuploids are as numerous as euploids among tetraploid derivatives. Probably functional gametes are restricted in number to  $2n \pm 1$ , although it is possible that the number 22 was obtained from  $10 + 12$  or  $9 + 13$ . At any rate, the variation in chromosome number of functional gametes in *rubra-foetida* is much more restricted than that found in functional gametes of hybrids in which the somatic number is greater. Hence the reaction system of a plant with smaller chromosome numbers is more susceptible to losses or gains than the reaction system of a plant with higher numbers, as shown previously in *Datura* tetraploids by Belling and Blakeslee (1924).

Considering the exceptional nature of B 18, it is probable that the chromosome rearrangement is no greater than that found in plants involving only a single pair. The chief distinction between the C chromosomes of the two parents rests on the presence or absence of the satellite. In certain backcross plants of ( $F_1$  x *rubra*) x self certain exceptions to expected results point to the possibility of interspecific crossing over. The somatic garniture of plant B 9 (fig. 3c) as well as B 18 (fig. 3a) may represent the effects of crossing over. In both plates one of the chromosomes designated *foetida* C is too long for a normal  $C_r$  chromosome, despite the fact that a *foetida* satellite is present. The length of the C chromosomes in these two cases suggests that of  $C_r$  chromosomes. In B 9 the satellited chromosome in the center of the plate is too long for  $C_r$  and the so-called  $C_r$  immediately to its right is entirely too short for a *rubra* C chromosome.

The question arises, is this cytological evidence of crossing over, or merely a result of fragmentation and attachment? The observed chromosome alterations may conceivably represent the former situation, because single cross overs between these two interspecific homologues would be expected to produce such alterations.

Consequently, it is probable that the third  $C_r$  chromosome of B 18 is partly or mainly *foetida* in constitution. Furthermore, the apparently extra A chromosome in the same plate could easily be an E chromosome with an attached segment, hence, indistinguishable from an A. There are good reasons for supposing this to be true, as will be discussed presently. In other words, it is possible that B 18 belongs genetically in that group of euploid plants possessing rearrangements in only one set of homologues.

Plant C 10 is the only plant classified as  $4n$  which proved unable to set seed, and since it had 31.2 per cent good pollen grains, and seemed in other respects to be an amphidiploid, its sterility was



Fig. 3. (a) B 18, most fertile plant of  $F_1$ , with a garniture of  $4n-C_1+C_2-D+A$  but with one so-called  $C_1$  too long for a normal  $C_1$ , (b) B 7 the second most fertile  $F_1$  plant with garniture  $-D+E$  (both B 7 and B 18 were used as parents for  $F_2$ ); (c) B 9 classified tentatively as a  $4n$  plant but with abnormal  $C_1$  and  $C_2$  chromosomes.

probably due to some non-genetic causes. This conclusion is strengthened from a consideration of B 12, also a  $4n$  plant with no rearrangements, which had 37 per cent good pollen, and which was observed

to have set seed before an untimely death. Therefore, it may be considered that the only sterile *euploid* plants in  $F_2$  are those in which rearrangements involve more than one set of homologous chromosomes. On the other hand, rearrangements in the eight A and six E chromosomes are undetectable, and if sterility in plants with only one rearrangement is accompanied by low pollen grain counts, such undetectable rearrangement in A's or E's could be inferred; e.g., B 16 and C 20.

Evidently departures from the amphidiploid constitution are limited in range if fertility is to be maintained, since it appears that the loss or gain of a single chromosome, or the rearrangement of more than a single pair of homologues contributes to both genetic and zygotic sterility. The question then arises, to what extent may improvements in the fertility of amphidiploids be expected where all the homologues from both genomes are capable of quadrivalent conjugation?

*Genetic analysis.*—The second feature of interest from the data of table 1 concerns the factor pairs nodding-erect bud position, and purple-yellow anther tubes. In a previous publication (Poole 1931) it was shown that the characters, nodding buds and purple anther tubes, come from *rubra*, and the characters, erect buds and yellow anther tubes, come from *foetida*. In the backcross of  $F_1$  to *rubra* × self the combined genetic and cytological analysis showed the following association between chromosomes and characters:

C<sub>1</sub>C<sub>2</sub>, nodding buds, purple anther tubes

C<sub>3</sub>C<sub>4</sub>, nodding buds, purple-tipped, yellow-bodied anther tubes

CrC<sub>r</sub>, erect buds, yellow anther tubes

In the  $F_2$  generation under discussion here, there were three plants, each having 19 chromosomes, in which no *rubra* C chromosomes were discernible; these are C 11, B 19, and C 19, in the order in which they appear in table 1. According to hypothesis all should have had yellow anther tubes and erect buds provided there were no inter-specific crossing over. Only two of the plants, however, had yellow tubes, and none of the three had erect buds. No other  $F_2$  plants had yellow anther tubes except these two, although seven had erect buds. From the somatic garnitures it is evident that all three of these plants have one C<sub>r</sub> longer than normal, hence as far as the cytological evidence goes the original hypothesis, that the factors for these two pairs of characters are carried in the C chromosomes of both species, has not been seriously damaged. In view of the numerous opportunities

for chromatin interchange it is evident that the classification of certain questionable chromosomes in hybrid derivatives on the basis of their resemblance to the parental chromosomes is no more than a convenience.

A genetical demonstration of crossing over involving eight homologous strands is entirely too difficult to be made with a population of only 44 plants. Furthermore, crossing over may have occurred in the  $F_1$  as well as in the  $F_2$  amphidiploid. If the allelomorphic pairs are considered separately, however, there is surprisingly close agreement between the observed and expected ratios for a tetraploid.

| SEGREGATION FOR ANTHER TUBE PATTERN |              |              |              |       |
|-------------------------------------|--------------|--------------|--------------|-------|
|                                     | Purple tubes | Tipped tubes | Yellow tubes | Total |
| Expected.....                       | 1.22         | 41.56        | 1.22         | 44    |
| Observed....                        | 2            | 40           | 2            | 44    |

| SEGREGATION FOR BUD POSITION |              |            |       |
|------------------------------|--------------|------------|-------|
|                              | Nodding buds | Erect buds | Total |
| Expected.....                | 33           | 11         | 44    |
| Observed .....               | 37           | 7          | 44    |

If we assume that for anther tube pattern, only PPPP gives purple tubes and only pppp gives yellow tubes, and that all heterozygous constitutions are tipped, then the expected ratio of 1:34:1, very well fits the observed 2:40:2 ratio. Likewise for bud position, which is known to have a quantitative interaction in diploid derivatives, if we assume that one E in the constitution Eeee is insufficient to express dominance, then the expected genotypes will be grouped into a phenotypic ratio of 27 nodding to 9 erect. In the present instance the observed ratio of 37:7 fits fairly well, giving a probability of 83.5 per cent that a deviation of 4 is due to chance.

The two species *C. rubra* and *C. foetida* are good species from every point of view. Taxonomists of the old school, who view with disapproval the efforts of cyto-geneticists in the field of experimental evolution, seem to imagine that Mendelian character differences are not concerned in the evolution of plant species. Therefore the fact that two pairs of specific characters, such as the ones discussed above, should segregate as they do in an interspecific hybrid is good evidence that evolution is partly concerned with Mendelian factors.

THE  $F_4$  POPULATION

This population comprised three progenies, each derived from a single  $F_3$  plant. Two of the parents chosen were the most fertile of the  $F_3$  plants, B 7 and B 18 (figs. 3b and 3a). The third plant, B 11 (not illustrated), was chosen because it had 21 chromosomes of the constitution  $4n + A$ . The number of seeds from these three progenies that germinated was about 300, and since root tips were to be fixed from all, no other  $F_3$  plants could be included as parents for lack of convenience in their proper investigation. The three parents exhibited sufficient diversity in their chromosomes to present a fair range of the potentialities of a larger group of possible  $F_3$  parents. The principal aims were to produce (a) stable derivatives of as nearly complete fertility as possible, (b) to note to what extent further rearrangements of chromosomes might result in further enhanced fertility, and (c) whether rearranged chromosome garnitures would continue in a direction already initiated, or duplicate the results observed in  $F_3$ .

The satisfactory utilization of pollen determinations for indicating fertility in  $F_3$  suggested a more prominent use of this study in  $F_4$ . Incidentally a study was made of the production of pollen in several pure *Crepis* species flowering in the winter months preceding the flowering of the  $F_4$  population. From the latter study information was secured indicating that fluctuation in the production of aborted pollen was dependent on physiological adjustments to flowering and senescence. In pure species the deviation in aborted pollen grains was extreme at the beginning of the cycle, but was shortly followed by a steady production of the amount of bad pollen expected from the genetical constitution. This, of course, would differ for different species or hybrids (Poole 1932). In order to provide a check on pollen production, therefore, two determinations of pollen percentages, three weeks apart, were made in all  $F_4$  plants. On the whole the two determinations agreed, certain exceptions being found where the experimental error was large, but the correlation between the two determinations was of the order of  $0.7208 \pm 0.0388$ .

In  $F_3$  those plants showing 30 per cent or more good pollen were isolated from cross-pollination in mosquito-netting cages. As a result of this practice the caged plants were injured during a spell of several days of very hot weather, while the uncaged plants were not

affected, as the data presented in that study show. As soon as the injury was noticed the cages were removed, but this was too late to benefit any of the more highly fertile plants but one, 7.63, which was the last of these to flower.

Extreme diversity was noted among the plants of all three progenies. In rosette types alone scarcely any two plants were alike in any features, and no two were identical. Similarity was observed in only one feature which concerned the color of the setules found on the upper surface of the midribs of rosette leaves. In progeny Z 18, from B 18 of the preceding year, only five or six plants from a total of 125 reaching maturity showed any trace of color in the cell sap within the setules, all other plants being without color, regardless of whether or not the stems possessed anthocyanin. In progeny Z 7 from B 7, all the members of the progeny showed colored setules, whereas in progeny Z 11, from B 11, about half of the plants had colored and half of them uncolored setules.

A study of the degree of diversity in the population was possible for certain characters of a quantitative nature, such as percentage of good pollen, percentage of fertility, width of first flower heads when completely opened, and stature of plant when the first head flowered. This diversity is illustrated by the figures presented below for only two of these characters.

#### VARIABILITY OF $F_4$

|                          | Percentage fertility |            | Percentage good pollen |            |
|--------------------------|----------------------|------------|------------------------|------------|
|                          | Mean                 | Coef. var. | Mean                   | Coef. var. |
| All plants . . . . .     | 0.603                | 210        | 15.662                 | 61.6       |
| Caged plants . . . . .   | 1.169                | 103        | 27.641                 | 25.8       |
| Uncaged plants . . . . . | 0.492                | 253        | 13.303                 | 61.7       |

#### VARIABILITY OF $F_2$

|                      |       |     |        |      |
|----------------------|-------|-----|--------|------|
| All plants . . . . . | 0.966 | 183 | 27.477 | 35.6 |
|----------------------|-------|-----|--------|------|

Despite the injurious effects of caging, the caged plants not only exhibited a higher percentage of fertility but much less variability. The uncaged plants on the other hand showed the greatest variability and the lowest means. It will be noticed that the mean percentage of good pollen of the selected caged plants was practically the same as that for the entire  $F_2$  population, indicating that even the choicest members of the  $F_4$  population presented little basis for the hope of enhancing the fertility.



Since time to prepare root sections and to study the somatic chromosome garnitures of the entire 237  $F_4$  plants reaching maturity was not available, a sample of the entire population was selected in the following manner. The most fertile plants were selected as parents for the coming  $F_5$  and consequently knowledge of their somatic garnitures was required. In addition to these an equal number of infertile plants were chosen for examination. Such a choice would scarcely be expected to give a true picture of the entire population as regards total chromosome numbers, because the experience in  $F_3$  showed that fertility was practically restricted to plants possessing 20 chromosomes, whereas the less fertile plants would be expected to have as many with as without 20 chromosomes. This is approximately what was found, as may be seen below.

$F_4$  CHROMOSOME NUMBERS

| Progeny   | Parent chromosome Nos. | 19 | 20 | 21 | 22  | Total | Total progeny |
|-----------|------------------------|----|----|----|-----|-------|---------------|
| Z 7       | 20                     | 2  | 9  | 3  | ... | 14    | 83            |
| Z 11      | 21                     | 1  | 7  | 3  | 1   | 12    | 37            |
| Z 18      | 20                     | 2  | 8  | 2  | ... | 12    | 117           |
| Total ... | .....                  | 5  | 24 | 8  | 1   | 38    | 237           |

The proportion of plants with 20 chromosomes to the total number is greater than found in  $F_3$ , where there were as many having numbers other than 20, as with 20. The disproportion observed was doubtless due to the method of selecting the plants to be examined.

The correlation of the somatic garnitures with the percentage of fertility confirmed the results obtained in  $F_3$ , except that there were only four plants out of the 24 euploids showing a reconstituted amphidiploid garniture. In  $F_3$  half the euploids exhibited an amphidiploid garniture. It was again observed (see table 2) that the most fertile group of derivatives was one exhibiting rearrangements in only one pair of homologues. This time, however, the information was more definite than in  $F_3$ , for here it appears that one particular pair of homologues is more important than the others, i.e., D/E<sup>2</sup>. Furthermore, a glance at the garniture formulae of  $F_3$  and  $F_4$  shows that the unique D chromosome from *rubra* is missing more frequently than any other chromosome. This may be shown more conveniently by the following list of missing chromosomes for  $F_3$  and  $F_4$ . In compiling this list it was necessary to make one assumption, which seems well justified,

TABLE 2  
GENETIC AND CYTOLOGICAL DATA OF  $F_4$   
20 CHROMOSOME PLANTS

| Plant No. | Per cent first week | Good pollen third week | Per cent fertility | No. of achenes | Formulae                     |
|-----------|---------------------|------------------------|--------------------|----------------|------------------------------|
| 7.70*     | 44.1                | 34.8                   | 1.77               | 66             | } ..... $4n$                 |
| 11.18     | 20.7                | 20.3                   | 1.74               | 104            |                              |
| 11.35     | 4.8                 | .....                  | 0                  | 0              |                              |
| 18.38     | 16.7                | 27.0                   | 2.66               | 145            |                              |
| 7.22      | 10.6                | 9.7                    | 0.81               | 26             | } ..... $-C_t + C_r$         |
| 7.43      | 19.8                | 21.8                   | 0.70               | 13             |                              |
| 11.3*     | 7.3                 | 8.5                    | 0.08               | 3              |                              |
| 5*        | 29.8                | .....                  | 0                  | 0              |                              |
| 14        | 12.4                | 19.7                   | 0.73               | 19             |                              |
| 18.5*     | 31.4                | 27.3                   | 4.17               | 149            |                              |
| 11.18     | 8.0                 | 5.6                    | 0.30               | 8              | ..... $-C_r + C_t$           |
| 7.21*     | 44.9                | 33.3                   | 3.25               | 33             | } ..... $-D + E$             |
| 28*       | 33.0                | 24.3                   | 4.46               | 223            |                              |
| 73        | 17.8                | 23.8                   | 8.95               | 356            |                              |
| 11.17*    | 44.0                | 22.8                   | 3.05               | 105            |                              |
| 18.47*    | 40.6                | 33.3                   | 3.69               | 116            |                              |
| 18.28     | 13.7                | 19.0                   | 0.77               | 5              | } ..... $-2C_t + 2C_r$       |
| 71        | 14.4                | 32.9                   | 4.62               | 119            |                              |
| 7.64      | 21.8                | 21.7                   | 1.11               | 47             | } ..... $-2D + 2E$           |
| 74*       | 37.2                | 27.4                   | 2.53               | 116            |                              |
| 63        | 41.3                | .....                  | 7.27               | 320            | ..... $-C_r + C_t - D + E$   |
| 18.29     | 8.3                 | 7.2                    | 0.08               | 3              | } ..... $-C_t + C_r - D + E$ |
| 33*       | 33.7                | 30.3                   | 1.05               | 34             |                              |
| 20        | 21.9                | 23.0                   | 0.13               | 3              |                              |

## 19 CHROMOSOME PLANTS

|       |      |      |      |   |                        |
|-------|------|------|------|---|------------------------|
| 11.38 | 0.9  | 5.0  | 0    | 0 | ..... $-C_r$           |
| 7.26  | 9.9  | 11.2 | 0    | 0 | ..... $-A - C_t + C_r$ |
| 37    | 16.4 | 13.4 | 0    | 0 | ..... $-C_r - D + E$   |
| 18.6  | 18.1 | 12.7 | 0.34 | 7 | ..... $-A - D + E$     |
| 16    | 5.9  | 1.7  | 0    | 0 | ..... $-C_t + C_r - D$ |

## 21 CHROMOSOME PLANTS

|        |      |       |      |     |                                  |
|--------|------|-------|------|-----|----------------------------------|
| 11.9   | 8.5  | 6.7   | 0    | 0   | } ..... $+A$                     |
| 29     | 21.1 | 22.2  | 0.79 | 17  |                                  |
| 7.30   | 26.3 | 24.2  | 7.58 | 308 | } ..... $-D + 2E$                |
| 18.98* | 41.9 | 28.4  | 2.31 | 54  |                                  |
| 7.9    | 9.3  | 8.6   | 0.52 | 9   | ..... $-2C_t + 2C_r + E$         |
| 17     | 5.3  | 1.2   | 0    | 0   | ..... $-C_r + C_t - D + E + A$   |
| 18.13  | 3.7  | 4.3   | 0.03 | 1   | ..... $-C_t + C_r - D + 2E$      |
| 11.32  | 6.5  | ..... | 0    | 0   | ..... $-2C_t + 2C_r - E + D + A$ |

## 22 CHROMOSOME PLANT

|       |   |     |   |   |                          |
|-------|---|-----|---|---|--------------------------|
| 11.25 | 0 | 7.6 | 0 | 0 | ..... $-D + E + A + C_r$ |
|-------|---|-----|---|---|--------------------------|

\*Caged plants.

viz. the inclusion of D as an extra chromosome in a euploid plant indicates a loss of its homologue, E<sup>2</sup>.

MISSING CHROMOSOMES

|                | D        | E <sup>2</sup> | C <sub>r</sub> | C <sub>t</sub> |
|----------------|----------|----------------|----------------|----------------|
| F <sub>3</sub> | 20       | 2              | 11             | 7              |
| F <sub>4</sub> | 22       | 1              | 5              | 19             |
|                | <hr/> 42 | <hr/> 3        | <hr/> 16       | <hr/> 26       |

A tentative explanation of this situation is that D is the smallest chromosome and its loss is less disturbing to gametic viability. Nevertheless, no zygote is viable unless compensation for its loss is made by the substitution of its homologue E<sup>2</sup>, except in the case of  $4n-1$  plants. This observation applies to all other chromosomes as well, for in all 20 and 21 chromosome plants examined the only viable zygotes which succeeded in reaching maturity exhibit the substitution of a homologous chromosome for the loss of any given chromosome. A total of thirteen  $4n-1$  plants from both generations is a striking exception to this observation, unless we make reservations for 18.20 shown at the bottom of table 2, in which two missing D's are compensated by one E and what appeared to be one A. The parent of the progeny Z 18 was B 18 of F<sub>3</sub>, for which it has already been argued that the ninth A was an E with an attachment on one arm, consequently in 18.20 the extra "A" was probably a compound E<sup>2</sup>.

In figure 4 are illustrated three garnitures on which are shown unique chromosomes, each indicated by an arrow. This adds three more to the three already shown in figures 2a, 2b, and 3c. Such chromosomes have never been seen in the somatic cells of *C. rubra* or *C. foetida*. For obvious reasons these newly constituted chromosomes are C, D, or E, since they are the only ones in which alterations would be striking enough to lend assurance to their detection. Deficient A chromosomes would doubtless often be taken for E chromosomes, while attached E's might be taken for A's; but deficiencies in E's are represented by two examples, 18.28 and 18.98 (fig. 4a and c). No attachments to A's were noted with certainty, although several times their presence was suspected but could not be definitely identified in enough cells owing to the tendency of these longer chromosomes to twist and recurve off the equatorial plate. Plant B 9 (fig. 3c) has already been referred to as illustrating one

satellited  $C_7$  too long for a normal chromosome, and one unsatellited  $C_7$  too short to be normal. Other examples have already been mentioned, especially when discussing the genetic evidence for anther tube pattern and bud position. Here the presence of longer than normal  $C_7$  chromosomes was pointed out, although the plates are not



Fig. 4. Metaphase figures from three  $F_1$  plants each showing a deficient chromosome: (a) 18.28, a euploid with a deficient E, formula  $-2C_7+C_7$ ; (b) 7.30, an aneuploid with a deficient D; formula  $-D+2E$ ; (c) 18.98, an aneuploid with a deficient E, formula  $-D+2E$ .

illustrated in the included drawings. The most readily detected alterations are those occurring on D's, three included illustrations showing a deficiency (fig. 4b), an attachment to the body of the chromosome (fig. 2a), and a loss of the satellite in a plant possessing 3 D's, the other two of which were entirely normal (fig. 2b).

In view of the generally random nature of chromosome assortment in these derivatives, it is not surprising that  $F_1$  plants like B 7

and B 18, each of which lacked one D chromosome, should have produced progeny possessing two D's. This situation was observed five times out of a total of fourteen plants in progeny Z 7, and four times in progeny Z 18 out of a total of twelve plants.

**Fertility.**—In both  $F_2$  and  $F_4$  fertility is practically restricted to plants possessing the euploid chromosome number which, as we have seen, is maintained in the majority of cases not through a transmitted or reconstituted amphidiploid garniture, but as a result of rearrangements often quite complicated. It is remarkable, then, that the less complex rearrangements produce the more fertile combinations. This is most evident when rearrangements involve the single homologous pair D/E<sup>2</sup>. It is also seen from table 2, plant 7.63, where a second pair is involved, and also in  $F_2$  in B 18 where two pairs were involved, one of them in all cases being D/E<sup>2</sup>. The influence of D/E<sup>2</sup> is especially noticeable in the only two  $F_4$  aneuploid plants exhibiting fertility, 7.30 and 18.98 (figs. 4b and 4c) both of which have twenty-one chromosomes and the formula  $4n - D + 2E$ . In these two plants there is an extra E chromosome but at the same time there is one deficient chromosome in each plant. Because of the deficiency the nuclear balance has not been greatly upset by the addition of a single E chromosome.

The three most fertile plants of this population are three plants which were not caged, because in two of them, 7.73 and 7.30, the first pollen determination failed to show 30 per cent or more of good pollen grains. Although 7.63, the third most fertile plant, did show over 30 per cent good grains, it was the plant mentioned above which had its cage removed when the injury caused by overheating was first noticed. All caged plants which were studied for their somatic garnitures are indicated in table 2 with an asterisk, and it can be seen that their high pollen counts promised a higher degree of fertility than was observed. In the study of the pollen grains in relation to fertility, previously alluded to, it was shown that the caged plants as a group were the only plants to show no correlation between good pollen and fertility.

## DISCUSSION

There have now been reported in the literature of the past six years an aggregate of more than twenty experimentally verified amphidiploid hybrids, some of which were known as far back as 1880, although their true nature was not understood. Some of the investigators fail to report on fertility, but most of them have reported as to the regularity or irregularity of meiosis. From the list there appear to be ten which are as fertile as good species:

1. *Anemone sylvestris* x *A. magellanica* (Janczewski 1889) ?
2. *Aesculus hippocastanum* x *A. pavia* (Skovsted 1929)
3. *Fragaria bracteata* x *F. helleri* (Ichiyama 1926)
4. *Euphanus sativus* x *Brassica oleracea* (Karpechenko 1928)
5. *Rosa Wilsoni* (Blackburn and Harrison 1924)
6. *Nicotiana digluta* (Clausen & Goodspeed 1925)
7. *Nicotiana tabacum* x *N. rustica* (Rybin 1927)
8. *Aegilops ovata* x *Triticum dicoccoides* (Tschermak and Bleier 1926)
9. *Spartina townsendii* (Huskins 1931)
10. *Primula kewensis* (Newton and Pellew 1929)

In view of the fact that Janczewski's probable amphidiploid was not examined cytologically, there may be some question raised as to its inclusion in the species-like group. Certain others, now to be considered, are only fairly fertile, or show some amount of segregation in their progeny. Many, however, were reported to be highly irregular, or even entirely sterile:

11. *Digitalis ambigua* x *D. purpurea* (Buxton and Newton 1928)
12. *Triticum vulgare* x *Secale cereale* (Levitsky and Benetzkain 1929)
13. *Nicotiana paniculata* x *N. rustica* (Lammerts 1931)
14. *Solanum nigrum* x *S. luteum* (Jorgensen 1928)
15. *Crepis capillaris* x *C. dioscoridis* (Babeock & Navashin 1930)
16. *C. capillaris* x *C. tectorum* (Hollingshead 1930)
17. *C. rubra* x *C. foetida* (Poole 1931)
18. *Saxifraga potternensis* (Marsden-Jones 1930)

In the group 11-18, five are reported to show the presence, with variable frequency, of quadrivalent chromosomes in meiosis, and one other occurs among the list in which a tetraploid plant appearing in one of the parent species showed quadrivalents, although the number of pollen mother cells examined in the amphidiploid was insufficient to show whether or not the phenomenon existed (Jorgensen 1928). Despite the lack of detailed data reported along with the announce-

ments, it is clear that there are many different kinds of amphidiploids as to behavior; and certainly several different categories of origin have been reported.

The presence of quadrivalent chromosomes in species hybrids raises a question of correct terminology. Viewed solely from the standpoint of cytology, objections could be raised against calling any hybrid an amphidiploid if quadrivalents were observed. In such a case it could be argued that the occurrence of quadrivalents denotes a tetraploid rather than an amphidiploid condition. On the other hand, as soon as the taxonomic view is considered a new complexion is put on the matter. If both parents were unquestionably good species, possessing numerous character contrasts, in addition to obviously distinct somatic garnitures, then a  $4n$  hybrid between two such species is undeniably an amphidiploid. This question at least shows the desirability of considering the matter from more than one viewpoint.

Those amphidiploids which behave with the regularity of good species are undoubtedly representative of types which have functioned in producing genera wherein most of the species exhibit chromosome numbers in arithmetical series. Some genera appear to have evolved exclusively by this method, whereas others show only occasional species which probably originated through amphidiploidy. Although all of them are characterized by the possession of a complete diploid complement from both parents of the preceding generation, they have had different methods of origin, and exhibit different classes of behavior. Among the different ways in which these hybrids originated may be noted two main methods, (a) non-reduction of gametes, and (b) somatic duplication. Most of them have been reported to originate through the non-reduction of gametes, but among those originating by somatic duplication three subcategories exist: (a) bud variation, as in *P. kewensis*, (b) callose shoots, as in *S. nigrum* x *S. luteum*, and (c) endoduplication, as in *Nicotiana digluta*.

The evidence of quadrivalent chromosomes in five or six of the amphidiploids in the above list, and especially in *rubra-foetida*, makes it clear that much of the meiotic irregularity observed is due to genetic and cytological homology between the parent species. Such homology undoubtedly delays or altogether prevents the operation of amphidiploidy as an agency in evolution. Where homology is at a minimum, rearrangement of chromosome complements leading to the formation of harmonious reaction systems is ultimately possible and

will occur, as indicated by the tendency of *Nicotiana rustica-paniculata* in this direction. On the other hand, where homology is at a maximum, perhaps no amount of rearrangement will avoid disjunctional irregularities. Hence there are at least three kinds of amphidiploids possible: (a) no interspecific pairing, with regular maturation divisions; (b) a minimum amount of interspecific pairing, with rearrangement products leading to stable derivatives; and (c) a maximum amount of interspecific pairing, with probably no rearrangement products.

In the present state of this investigation it is difficult to say how the *rubra-foetida* hybrid will eventually behave. It is clear that thus far little ground exists for believing that stability will be reached. The hope that selection of the most highly fertile plants would result in the isolation of strains with gradually increasing fertility appears, from existing evidence, to be abortive. The garniture rearrangements of  $F_3$ , which were most fertile continued to assort at random in  $F_4$ , producing some of the same types, as well as many others, none of which produced any improvement in percentage of good pollen. Furthermore, in both generations the maximum fertility occurred only in euploid plants, and in such a way as to suggest restriction of fertility to a very narrow departure from the amphidiploid garniture. This departure was, in all the more fertile cases, of an unbalanced nature. In general, if a chromosome were missing from the  $4n$  set, the evidence indicated that the gamete involved had been fertilized by a gamete bearing an extra interspecific homologue, otherwise zygotic lethality resulted. This was true of all plants, euploids and aneuploids. But in  $4n-1$  aneuploids there was necessarily one, although only one, uncompensated loss, regardless of other rearrangements, and in  $4n+1$  aneuploids there was one, and only one, extra homologue. There were two  $4n+2$  plants, one in each generation, B 21 and 11.25. In both cases there were *two* extra homologues regardless of other rearrangements.

In view of this evidence the limits of gametic and zygotic viability may be inferred. From the general nature of the evidence it is probable that viable gametes carried only the numbers  $2n$ , and  $2n$  plus or minus 1 chromosome.

The evidence for zygotic viability is more definite. In *euploids* zygotic viability is apparently limited by (a) the necessity of a missing chromosome of a  $2n-1$  gamete meeting an extra homologue of the same set in a conjugating gamete, which must be  $2n+1$ ; or (b) by



gametes with the number  $2n$ , but with an extra chromosome of one set and a missing chromosome of another meeting a gamete with a missing chromosome of the first set and an extra chromosome of the second set.

In *aneuploids* the same compensation is required, as shown by the evidence when rearrangements have involved more than one homologous set. The difference in zygotic viability between euploid and aneuploid plants, however, is that in aneuploids of the type  $4n - 1$  one member of a given set is missing and in the type  $4n + 1$  one member of any set is extra, all other losses being compensated. In  $4n + 2$  plants one member of any two sets may be extra or missing, although no  $4n - 2$  plants have yet been observed.

In regard to zygotic fertility rather definite conclusions may be drawn also, for the most fertile zygotes of both generations were those in which rearrangements involved a lack of just one D chromosome, which represents the loss of the smallest possible amount of chromatin material. This quantitative feature of chromatin loss is emphasized by the fact that the two fertile  $4n + 1$  plants, 7.30 and 18.98, had chromosomal deficiencies which balanced the addition of one extra chromosome.

Irregularities of the type occurring in *Pisum*, *Oenothera*, and *Datura* are still possible here in view of the discovery of newly constituted chromosomes, as illustrated in figures 2a, 2b, 3c, 4a, 4b, and 4c. Furthermore, we have here evidence of the kind of chromosomal changes which differentiate the majority of *Crepis* species from one another. The majority of *Crepis* species have the same chromosome number, four pairs. Yet the species are all good taxonomically, and in addition to their factorial differences, most of them show slight alterations in shape or size of homologous chromosomes, of the kind here considered.

In view of these facts, investigations of *rubra-foetida* amphidiploid derivatives will be continued with progenies of large numbers. Either stable derivatives will eventually be found, or they will not. In case they are found the advantages of small chromosome numbers, and a certain amount of reliable chromosome individuality, will throw light on important evolutionary problems common in the plant kingdom—advantages not found in just this combination in less favorable material.

Even though no stable derivatives are eventually found from the amphidiploid here investigated, this will not invalidate the hypothesis

that the American species of *Crepis*, having a base number of  $n = 11$ , originated through amphidiploidy. As has been pointed out (Babcock and Navashin, 1930) the assumed amphidiploid hybrids most probably originated from crosses between species having  $n = 4$  and  $n = 7$ . All of the known species with these chromosome numbers are sufficiently distant in their relationship that no meiotic irregularities would prevent full fertility in their amphidiploid hybrids.

## SUMMARY

1. In the first selfed generation of derivatives from an amphidiploid plant of *C. rubra* x *C. foetida* the chromosome numbers were roughly one-half euploid and one-half aneuploid. The aneuploids were equally divided between  $4n - 1$  and  $4n + 1$ , whereas the euploids were equally divided between those having no rearrangements in the  $4n$  set, and those exhibiting various degrees of rearrangement from that set.

2. The second selfed generation from three plants of the first generation was not completely examined cytologically, but those examined indicated that the range in chromosomal distribution was analogous to that found in the first. Since none of the three parents was amphidiploid in garniture, however, the number of reconstituted amphidiploids was not as high as before, but the fact that they existed is indicative of the random nature of chromosome assortment.

3. Fertility in both selfed generations was almost entirely restricted to euploid plants, and among these it was especially marked in plants having chromosomal rearrangements involving but one pair of interspecific homologues, D/E<sup>2</sup>. There was no tendency in plants having garnitures that were completely *rubra* or completely *foetida* for any homologous sets to be fertile.

4. The only aneuploids exhibiting any degree of fertility were two having rearrangements involving the same set of homologues, D/E, and these were both of the formula  $4n - D + 2E$ . Furthermore, these two fertile  $4n + 1$  plants each had, in addition to one extra chromosome, one deficient chromosome, thus balancing the additional amount of chromatin matter.

5. The more fertile plants in both generations had garnitures of an unbalanced nature, even in euploids. But these most fertile deriva-

tives displayed very low fertility, never exceeding 10 per cent, which indicates that stable races are not likely to be derived from this amphidiploid.

6. The evidence for all plants, euploid or aneuploid, suggested that viability of the zygote was contingent on the compensatory presence of the interspecific homologues of any of the chromosomes lacking from one of the pairing gametes. Never was the absence of a particular chromosome compensated by the additional presence of a non-homologue.

7. In  $4n - 1$  aneuploids there was one, but only one, uncompensated loss; all other losses from one gamete being compensated by the presence of an extra interspecific homologue in the conjugating gamete. In  $4n + 1$  or  $+ 2$  plants extra homologues were present, but no losses were uncompensated.

8. The interspecific allelomorphous character pairs nodding-erect buds and purple-yellow anther tube patterns apparently segregated in the tetraploid ratio, even in the limited population available, corroborating the cytological evidence of random assortment.

9. Several well demonstrated cases of newly constituted chromosomes were observed in both selfed generations.

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# THE CHROMOSOMES AND RELATIONSHIP OF *CREPIS SYRIACA* (BORNM.)\*

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## INTRODUCTION

ALTHOUGH it has long been accepted that chromosome numbers are highly constant within species, a great many occurrences of deviation have been cited. It is very rarely, however, that a species is found in which variation is the rule rather than the exception. Such a discovery was reported by Hollingshead and Babcock (1930) in *Crepis*. While examining and comparing the chromosomes of a large number of species within this genus, individual plants of *C. syriaca* were found to possess from ten to thirteen chromosomes in somatic cells. The extra chromosomes, where present, were morphologically similar to one another, but different from any members of the basic group of ten. This condition in a species in which chromosome morphology is so well marked made it most favorable material for the investigation of supernumerary chromosomes. Other particularly advantageous characteristics of the species are the relatively low chromosome number and the fertility of plants with variant genomes.

The taxonomic relationship of *C. syriaca* is also of interest, particularly with respect to *C. alpina* L., of which it was formerly held to be a variety. Both species are in the same section of the subgenus *Barkhausia*, being most closely related to *Crepis rubra* L. and *C. foetida* L. of the same section (Babcock and Lesley, 1926, figs. 1 and 2, and table 2; Babcock and Navashin, 1930). In general appearance they are similar, yet there are many constant differences which serve to distinguish them as species. The more outstanding differences are tabulated as follows:

### *C. syriaca*

1. Habit low, spreading.
2. Herbage light green, lacking tomentum.
3. Radical leaves oblanceolate, acute, dentate or runcinate-pinnatifid or pinnately parted, the segments acute, dentate.
4. Cauline leaves laciniate at base.

### *C. alpina*

1. Habit tall, erect.
2. Herbage gray, tomentose.
3. Radical leaves obovate-oblong, obtuse, denticulate, sometimes with 3-4 irregular shallow lobes near apex.
4. Cauline leaves entire or denticulate at base.

\* *Crepis syriaca* (Bornm.) Babcock, MS.

*C. syriaca*—(Continued)

5. Heads nodding before anthesis.
6. Heads fully expanded in anthesis.
7. Ligules deep yellow.
8. Anther-tube appendages 0.7 mm. long, obtuse.
9. Achenes about 14 mm. long, not always sharply divided into two types.
10. Pappus 4.5–5.5 mm. long.
11. Plants flower about 108 days after planting.

*C. alpina*—(Continued)

5. Heads erect before anthesis.
6. Heads partly expanded in anthesis.
7. Ligules pale yellow.
8. Anther-tube appendages 0.4–0.5 mm. long, acute.
9. Achenes about 18 mm. long, of two distinct types, marginal and inner.
10. Pappus 6–7 mm. long.
11. Plants flower about 135 days after planting.

Although, in respect to their comparative morphology, there appears little doubt of the correctness of recognizing these plants as separate species, still their relationship is so close that a cytogenetical analysis is warranted. Individuals from the two species were therefore crossed and the later generations subjected to a cytological and less extensive genetical investigation.

These problems were suggested by Professor E. B. Babcock, to whom the writer is indebted for constant assistance and advice. It is also a pleasure to acknowledge the generous coöperation of Professor R. E. Clausen throughout the course of the work.

## MATERIALS AND METHODS

The majority of the *Crepis syriaca* plants used were derived from seed from herbarium material (accession number 1923). The original specimens were collected by M. Chijik, in Galilee, in 1924. Several plants were grown from this source by the writer in 1929 and from a study of these and their progeny most of the results here presented were obtained. Later accessions which were also used are numbers 3106, 3132, 3158, 3159, and 3167 from northern Lebanon near Hasroun, and 3168 from Antilebanon, near Baalbek. Accessions of *C. alpina* used for comparison and for hybridizing are numbers 1640 from the Copenhagen Botanic Garden, 1641 from Tiflis (indigenous in the Caucasus region), 2769 from the Leningrad Botanic Garden, and 2783 from the Charkow Botanic Garden.

Some variation in external morphology occurs among these accessions of *C. alpina*, especially between individuals of the Tiflis type and those from localities in Asia Minor, Kurdistan, western Persia, Transcaucasia, and Crimea. The former plants are sufficiently different to be classed as a subspecies of *C. alpina*. In habit they are somewhat more inclined to branch from the base of the plant, a character which is even more noticeable in *C. syriaca*. The radical leaves are acute, rather than obtuse, and are not so gray as the typical representatives of the species. The involucre bracts are purple-tipped, a character which is transmitted to all

F<sub>1</sub> plants in crosses with *C. syriaca*. The corollas are a brighter yellow color and the flower heads are, in general, smaller with shorter ligules. The achenes are of about the same length as typical *C. alpina*, but the beak is longer, in this respect also resembling *C. syriaca*. As will be pointed out later, these characters are of interest in connection with the possible origin of the latter species.

Root-tips obtained from plants five to ten days after transplanting to 6-inch pots were fixed in chrom-acetic-formalin solutions (cf. Hollingshead and Babcock, 1930). They were imbedded in paraffin and cut 8 $\mu$  thick. Buds were fixed in acetic alcohol (2 parts absolute alcohol, 1 part glacial acetic acid) and were examined from 70 per cent alcohol in iron-aceto-carmine.

Figures were drawn with the aid of a camera lucida at a magnification of 3750, using a Zeiss 1.8 mm. n.a. 1.3 oil immersion objective and Zeiss 12x compensating ocular.

### THE SOMATIC CHROMOSOMES OF CREPIS SYRIACA

Aside from the supernumerary units present in *C. syriaca*, the chromosomes of this species are virtually identical with those of *C. alpina* ( $2n = 10$ ). This fact coupled with the instability of plants with higher numbers is very good evidence that 10 is the basic diploid complement of *C. syriaca*.

A preliminary survey of the chromosomes of *C. syriaca* was presented by Hollingshead and Babcock (1930). Since this study was made, however, new chromosome numbers have appeared in the progeny of self-fertilized lines. Plants derived from accession number 1923, which had fourteen chromosomes or less, show five pairs of chromosomes consistently (see fig. 1b) resembling those of *C. alpina* very closely. In addition, there is a characteristic supernumerary unit which is present one, two, three, or four times in 11-, 12-, 13-, and 14-chromosome plants respectively. This extra chromosome appears as a small unit, possessing a subterminal constriction and bearing a large satellite at the proximal end (fig. 1a-X). In plants with fifteen, sixteen, and eighteen chromosomes respectively, the increase seems to involve more than this X chromosome. For example, in fig. 2b, showing a complement of fifteen chromosomes, there appear to be two extra E chromosomes and three X's (cf. fig. 1a). This may be due to a nondisjunction or nonconjunction in members of the basic complement and hence may be of rare occurrence. The chromosome groups containing sixteen and eighteen chromosomes (fig. 2c and d) do not show all the X chromosomes as pictured in figure 1a. There is little doubt, however, that the extra members are of this same type. Throughout the investigation, it was found that the difficulty in obtaining clear plates was greater in forms with higher numbers.

The drawings shown here were made with the primary purpose of depicting the variation in number, since only composite representations could indicate the complete morphology of the various groups.

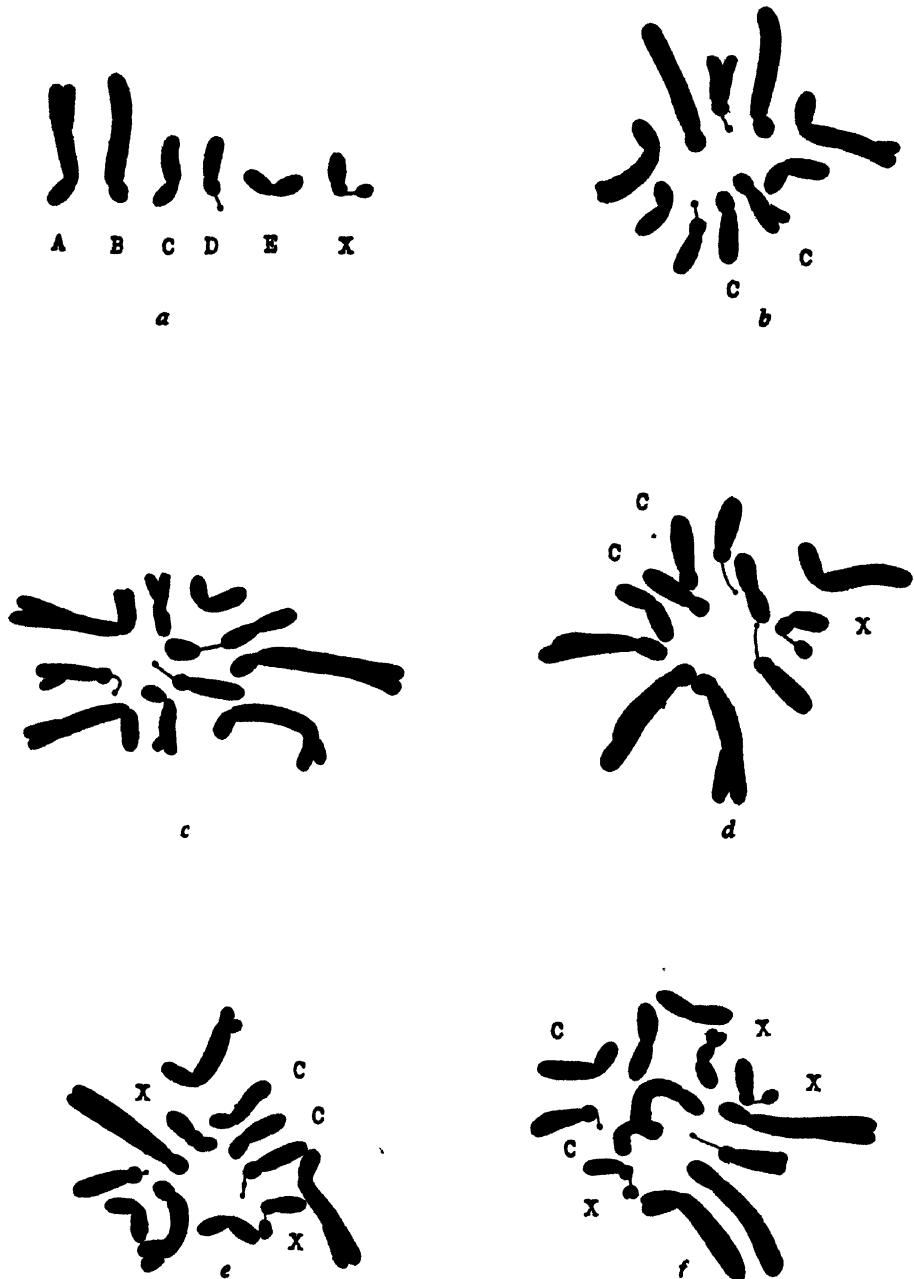


Fig. 1. *Crepis syriaca*. a, somatic chromosomes (haploid set); b, 1923 B6-2-8 (ten chromosomes); c, 3106 K1 (ten chromosomes)—note the large satellites on two non-homologous chromosomes; d, 1923 A 11-2-3 (eleven chromosomes), one supernumerary X chromosome; e, 1923 B7-1-3-1 (twelve chromosomes); f, 1923 B7-1-2 (thirteen chromosomes).



Fig. 2. *Crepis syriaca*. *a*, 1923 B7-3-1 (fourteen chromosomes); *b*, 1923 A 11-4-7 (fifteen chromosomes); *c*, 1923 A 11-4-6 (sixteen chromosomes); *d*, 1923 A 11-4-2 (eighteen chromosomes); *e*, 1923 A 11-4-6. Chromosomes of tetraploid cell, 32. The units in the lower right portion were in an adjacent section and some were obviously cut.

In the 16-chromosome plant obtained, a very high frequency of tetraploid cells occurred. The chromosomes of such a cell are shown in figure 2e. Those pictured at the lower right were from another section and some were obviously only a portion of chromosomes already drawn. A total of thirty-two units was counted in several cells and many others showed approximately the same number. Many binucleate cells were noted in this plant. Another unique circumstance was the presence of a large number of cells which exhibited profile views of metaphase chromosomes, with a consequent displacement of the usual regularity of cell layers. This was the only example of any abnormality in somatic mitosis which appeared during the study.

A much greater variability appeared in the cultures designated 3106. Figure 1c shows the chromosomes of a 10-chromosome plant from this accession. Instead of the usual five pairs ordinarily found in other 10-chromosome plants, a new abnormality appeared, involving the C and E chromosomes. Two large fragments of chromatin are attached to members of these pairs, upsetting the balance of the genom. All the plants derived from accession number 3106 appeared similar in their external morphology; yet there was not only a variation in chromosome number from plant to plant, but also variability in chromosome morphology within a single individual. So far no occurrences of variation in number of chromosomes among the cells of a single plant have been distinguished, nor do the two large pairs, A and B, ever become modified. In agreement with this observation Carothers (1917) suggests that, so far as she has observed, smaller chromosomes do not have a very pronounced effect when behaving irregularly, but that such irregularities associated with larger ones have a deleterious result. Among the varying chromosome groups in the cultures from accession number 3106, several cells were observed which contained a unit very similar in appearance to the X chromosome described earlier. It is conceivable that forms such as these may represent an intermediate step between normal 10-chromosome plants and the types described in the cultures grown from accession 1923.

## VARIATION IN CHROMOSOME NUMBER

Nearly all the accessions that have been studied up to the present time have shown variation, although the amount of material available in some was quite limited. Plants grown from various accessions have been found to exhibit chromosome variability as follows: (a) variation in number between plants—1923; (b) variation in morphology within plants—3106, 3132, 3167, 3158; (c) variation in both number and morphology—3159; (d) a single plant of accession number 3168 showed the normal ten chromosomes of *C. syriaca*.

It is of course entirely possible that further variations in chromosome

number may be found in accessions other than 1923, since this one was studied much more intensively than the others.

In 1929 a number of cultures were grown from open-pollinated seed collected from plants grown from original accession number 1923. Table 1 shows the results of chromosome counts of plants obtained from this source. Among the cultures classified as derived from 10-chromosome parents, several were grown from seed collected from plants of which the chromosome number had not been determined. However, from the situation in the progeny there was no doubt concerning their chromosome complements.

TABLE 1  
FREQUENCIES OF PLANTS HAVING DIFFERENT CHROMOSOME NUMBERS

| Parental plants  |                       | Frequencies of plants obtained with numbers of: |    |    |      |
|------------------|-----------------------|---|----|----|------|
| Number of plants | Number of chromosomes | 10  | 11 | 12 | 13   |
| 7                | 10                    | 81  | 2  | 6  | .... |
| 2                | 11                    | 5   | 12 | 2  | 1    |
| 2                | 12                    | 1   |    | 8  | 1    |
| 1                | 13                    |   | .  | 1  | 4    |

It is significant that, among the progeny, by far the greater number of plants had ten chromosomes. Since the parental plants were selected at random it is obvious that in the parental population 10-chromosome plants were more numerous than those with higher numbers. In each progeny group there was a marked tendency to reproduce the parental chromosome number. Among the progeny of 10-chromosome parents the variant numbers were 11 and 12; while the progeny of 11- and 12-chromosome parents varied in both directions from the parental number. In no plant was a complement of less than ten chromosomes found.

The foregoing results seem to indicate that certain lines have a greater tendency to vary than others. Thus, referring to table 2 of Hollingshead and Babcock (1930), the parent plant 1923-2 showed much more variation in its progeny than the plant or plants giving rise to culture 1923-A. From the results outlined here, it is altogether likely that plant 1923-2 had twelve chromosomes, while the parents of culture 1923-A had ten. Further similar evidence will appear later. Additional determinations of plants from accession number 1923 amplify the last line of this previously published table as follows:

|                            | Chromosome number |    |    |    |
|----------------------------|-------------------|----|----|----|
|                            | 10                | 11 | 12 | 13 |
| 27. 1923-2 Open-pollinated | 2                 | 3  | 8  | 5  |

In order to eliminate the possibility that fluctuations were brought about by crosses between plants which differ in chromosome number, individual selections were selfed to produce the later generations. Thus, except where specifically designated, the remaining data were obtained from self-fertilized lines.

TABLE 2

FREQUENCIES OF PLANTS (FROM SELFING) WITH DIFFERENT CHROMOSOME NUMBERS

| Year grown | Pedigree      | Chromosome number of parent | Frequencies of plants with numbers of: |      |      |      |      |      |      |      |
|------------|---------------|-----------------------------|--|------|------|------|------|------|------|------|
|            |               |                             | 10                                     | 11   | 12   | 13   | 14   | 15   | 16   | 18   |
| 1930       | A2-10.....    | 10                          | 2                                      | .... | .... | ..   | .... | .... | ..   | ..   |
| 1930       | A2-15.....    | 10                          | 5                                      | .... | .... | ..   | ..   | ..   | .... | .... |
| 1930       | B6-2.....     | 10                          | 8                                      | .... | .... | .... | .... | ..   | .... | ..   |
| 1931       | A2-10-2 ..... | 10                          | 3                                      | ..   | ..   | ..   | ..   | ..   | ..   | ..   |
| 1931       | A2-15-5.....  | 10                          | 9                                      | .... | .... | ..   | ..   | ..   | ..   | ..   |
| 1931       | B6-2-5 .....  | 10                          | 9                                      | ..   | ..   | .... | ..   | ..   | ..   | ..   |
| 1932       | A2-10-1 ..    | 10                          | 2                                      | 1    | ..   | ..   | ..   | ..   | ..   | ..   |
| 1932       | A2-15-5-2 ..  | 10                          | 5                                      | ..   | ..   | .... | ..   | ..   | ..   | ..   |
| 1930       | A11-2 .....   | 11                          | 1                                      | 5    | 4    | ..   | ..   | ..   | ..   | ..   |
| 1931       | A11-2-8 ..    | 11                          | ..                                     | 6    | 1    | 1    | ..   | ..   | ..   | ..   |
| 1930       | B7-3.....     | 12                          | ..                                     | ..   | 3    | 1    | 5    | ..   | ..   | ..   |
| 1931       | A11-2-1 ..... | 12                          | 3                                      | ..   | 4    | 2    | 1    | ..   | ..   | ..   |
| 1931       | A11-2-6 ..... | 12                          | 3                                      | ..   | ..   | .... | ..   | ..   | ..   | ..   |
| 1931       | B7-8 .....    | 12                          | 1                                      | ..   | 2    | ..   | ..   | ..   | ..   | ..   |
| 1931       | A11-4 .....   | 13                          | ....                                   | ..   | ..   | 2    | 3    | 1    | 2    | 1    |
| 1932       | A11-4-3 ..... | 13                          | 2                                      | ..   | 1    | ..   | 1    | ..   | ..   | ..   |
| 1932       | A11-4-4 ..    | 14                          | ..                                     | 1    | ..   | ..   | ..   | ..   | ..   | ..   |

It is unfortunate that larger numbers of plants could not be examined. This was often impossible because of the small amount of seed set by certain plants and the inviability of plants with particular somatic complements. In other examinations larger numbers would not have contributed any valuable information since the chromosome numbers could be predicted from previous determinations. In 1932 all the progeny from an 11-chromosome individual died after reaching the rosette stage. Only one survived until root-tips could be collected and these were so poor that an accurate determination was impossible. At the same time an entire culture of plants from a 10-chromosome parent succumbed. Apparently there was a factor influencing the physiology of these plants, making them more susceptible to slightly adverse environmental conditions. Other cultures, alternating with these, remained perfectly healthy.

Table 2 shows the frequencies of plants having different chromosome numbers in self-fertilized lines. In all the cultures derived from 10-chromosome parents, only one variant appeared. This 11-chromosome individual may have been the result of an accidental cross or of a mixture of seed, but at any rate a very high degree of constancy is revealed.



This result is only to be expected if ten is the "normal" somatic complement of the species. In the progenies obtained from open-pollinated plants, the majority of individuals have the same chromosome count as their parents. In selfed lines, however, this tendency grows progressively less marked with the increase in chromosome number. It is still quite noticeable in the 10- and 11-chromosome series, but in 12- and 13-chromosome lines the tendency is toward the production of still higher numbers. In the culture from an open-pollinated 13-chromosome plant, for example, four 13's and one 12 were obtained. When another 13-chromosome plant was selfed, individuals with thirteen, fourteen, fifteen, sixteen, and eighteen chromosomes were obtained. This, of course, may have been owing to differences in the parental plants. In general, there did not appear to be any tendency on the part of plants with larger numbers to return to the 10-chromosome type, although, when this condition was reached, it was virtually always maintained.

#### RELATION OF CHROMOSOME NUMBER TO EXTERNAL MORPHOLOGY

In respect to the external characters exhibited by the plant, the extra chromosomes appear to have small and inconsistent effects. In general, in plants having from two to eight supernumeraries, the flower heads are small and asymmetrical, and frequently have split ligules. The 10- and 11-chromosome individuals are usually normal in this respect although some individuals among the plants possessing eleven chromosomes have narrower ligules with longer teeth than in the normal forms (plate 12, figs. 2 and 3). There is also a tendency for plants with higher numbers to have broad, coarse leaves with margins which are merely toothed instead of margins more deeply cut, as in the 10-chromosome types. This alteration seems to depend more on the parental complement than on the number of chromosomes actually contained in plants with the type of foliage described. The difference is brought out clearly in the 1932 cultures. In the progeny of a 13-chromosome plant, two individuals appeared having ten somatic chromosomes. These were similar to normal individuals except in their broad radical leaves and generally more robust habit. There is here an indication that other changes besides gross morphological ones may be taking place in the chromosomes. As far as can be seen from a microscopic examination, the chromosomes appear identical in all 10-chromosome plants, regardless of their origins. On the whole, the 10-chromosome individuals are of a paler green color than are the plants containing supernumeraries, but there is sufficient variability to make a thorough color investigation of little value.

Another occasional abnormality is the development of an irregular rosette. This occurs in individual plants and is associated with eleven,

fourteen, and sixteen somatic chromosomes. In spite of their morphological irregularity, these plants show uniform reduction divisions and produce a high percentage of good pollen. No plants of this type occur in the lines characterized by the possession of ten chromosomes. In several of the irregular forms the rosette has two distinct centers of growth, giving to some of the leaves the appearance of being inverted.

Most of the cultures derived from 10-chromosome plants were consistently uniform in external morphology. However, in 1930 an interesting new type appeared among them. In two cultures, five small plants appeared, which were approximately 15 cm. high as compared with 45 cm. for their normal sibs. The contrast between these two types can readily be seen by a comparison of plate 12, figures 1 and 2. Little attention was given these plants at the time, since occasionally dwarfing occurs as a result of unfavorable growing conditions. In 1931, however, a culture of ten plants was grown from one of these variants, all of which were of the same dwarf habit. From another individual, in the same year, progeny were grown which showed segregation for this character. The tallest plant in the culture was 50 cm. in height, the smallest about 15 cm. The nine plants of this culture which reached maturity could readily be grouped into two size-classes of 7 tall : 2 dwarf. Although these numbers are far from adequate as statistical data, it seems altogether likely that the first selected plant referred to must have been homozygous, and the second, heterozygous for the character. Hence, it is probable that an intermediate height condition appears in the heterozygote, as it was a relatively small plant that gave rise to the segregating population. In 1932 a culture was grown from one of the dwarf segregants, of which all the plants were dwarf. In order to determine the nature of the genes associated with the new character, crosses were made between the two types. The resulting seeds were inviable.

### MEIOSIS IN *CREPIS SYRIACA*

A study of microspore mother-cells in this genus is attended with more than ordinary difficulty. The chromosomes are very large and irregular in shape, making an analysis of the meiotic behavior not an easy matter. The majority of cells in smear preparations present a profile view of the metaphase plate and the total number of cells obtained from one floret is small.

The 10-chromosome plants showed five pairs at I M in all preparations where a definite determination was possible. In other *Crepis* species which have been examined, a small amount of variation has been seen, so that it is very likely that a similar condition would occur here if the studies were prolonged. In good preparations a large number of cells were seen which had the appearance of the one represented in figure 3a. Figure 3b

shows a drawing of a plate typical of the 11-chromosome forms. Six units were consistently present involving five bivalents and a univalent. Usually the bivalents behaved normally, although at times one of these units was seen to divide precociously. The univalent divided at the first division and at the second the halves separated to either pole. Thus, apparently 5- and 6-chromosome gametes were regularly produced by this type of plant. On this basis an 11-chromosome plant when selfed should yield 10- 11- and 12-chromosome progeny in the ratio of 1:2:1 if a similar situation exists in the embryo sac. Table 2 shows, however, that the distribution is not of this type.

The discrepancy between observed and expected frequencies of 10-, 11-, and 12-chromosome plants derived from 11-chromosome parents may result from a qualitative difference in the chromosomes involved. There seems to be a definite tendency for plants with more than five pairs of chromosomes to yield progeny with more supernumeraries than they possess. This fact leads to the suggestion that the supernumerary unit may possess genetic material which is of considerable value in development. However, 10-chromosome plants show little or no inclination toward yielding progeny with larger numbers of chromosomes. Let us assume that the X chromosome arose from a reorganization of the chromatin of the basic five pairs by fragmentation and attachment. Then the portion of genetic material contributed would be duplicated in the genomes of plants with more than ten chromosomes. If this portion were associated with increased vigor or viability, it might reasonably be expected that 6-chromosome gametes of an 11-chromosome plant would function at the expense of 5-chromosome gametes and hence would lead to a preponderance of higher-numbered forms.

In several preparations there was an aberrant behavior of the divided univalent at I A in 11-chromosome plants. Instead of the halves going to opposite poles, they were both seen in close proximity to one end of the cell even after the other chromosomes had merged and were no longer distinguishable as separate units (fig. 3e). No micronuclei were apparent, however, so these must have been incorporated in the daughter nuclei at the second division.

A greater amount of irregularity could be seen in 12- and 14-chromosome plants. In spite of this, a remarkably high percentage of complete pairing was evident. In 12-chromosome forms only minor irregularities were noted and these occurred infrequently. In nearly all cells which presented clear views, six bivalents could be distinguished (cf. fig. 3c). Occasionally a precocious division of a bivalent occurs, or lagging of the univalents after the bivalents have split.

In 14-chromosome plants (fig. 3d) a peculiar condition was revealed. Some flower heads showed a very high percentage of regularity in meiosis, seven bivalents being regularly formed. In other florets on the

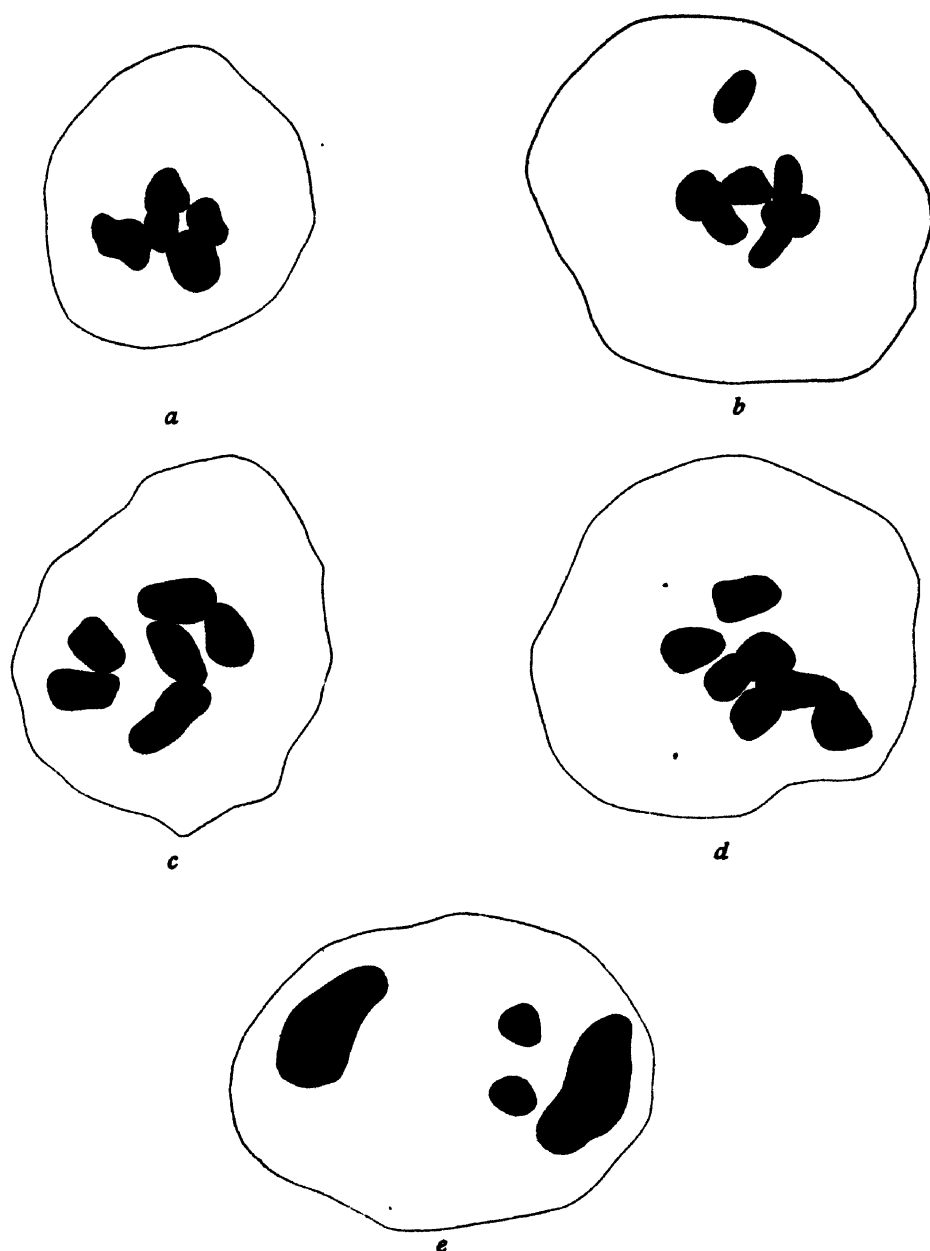


Fig. 3. *Crepis syriaca*. *a*, 1923 B6-2-5 (ten chromosomes forming five bivalents); *b*, 1923 A2-10-1-1 (eleven chromosomes forming five bivalents plus one univalent); *c*, 1923 A 11-2-1-7 (twelve chromosomes forming six bivalents); *d*, 1923 A 11-4-1 (fourteen chromosomes forming seven bivalents); *e*, 1923 A 11-4-4-1 (eleven chromosomes; telophase showing both halves of divided univalent at one pole).

same plant there was great irregularity. Chromatin masses appeared in place of the usual independent units. These in many preparations were accompanied by small fragments and division seemed to be brought about by a simple fission of the general mass. This highly abnormal type of division would account for the large percentage of poor pollen formed by such plants.

In maize, Randolph found all types of pairing among the extra chromosomes. An occurrence was also reported of pairing between one of the supernumeraries and one of the normal bivalents. There was a difference in the staining reaction of the extra univalents, the latter being somewhat darker than the rest of the complement.

### EFFECT OF CHROMOSOME NUMBER ON POLLEN FORMATION AND FERTILITY

Although there appears to be no clear-cut correlation between chromosome number and morphology in *C. syriaca*, an excess of chromatin does have a marked effect on pollen formation and fertility. Furthermore, the amount of excess chromatin has a greater effect apparently than the chromosome unbalance. This can readily be seen by an examination of table 3, which shows the percentage of undeveloped pollen grains in different chromosome-number classes. Since some of the figures were obtained, it has been found that occasionally the first flower formed yields highly aberrant results. For this reason there have been omitted a few plants which were obviously of this type and which would consequently give misleading average values for the different classes.

The plant numbers marked with an asterisk refer either to individual plants or to an average of two, which occurred in populations derived from plants with chromosome numbers other than their own. The rest were derived from plants with the same number, as is indicated in the second column. Only a few 14-chromosome plants were available for pollen counts and these showed from 30 per cent to 50 per cent undeveloped grains.

These figures are not conclusive, since a comprehensive study of pollen development and fertility was not intended. The table does show, however, that there is a marked difference between 10-chromosome forms and those which contain larger chromosome complements. It might well be expected that 12-chromosome individuals, having a balanced chromosome condition, would show a higher percentage of good pollen than the 11's and 13's. This does not prove to be the fact. There seems to be a definite tendency for the higher-numbered individuals to have a larger percentage of undeveloped pollen, irrespective of whether the chromosome number is odd or even.

TABLE 3

## CHROMOSOME NUMBERS AND POLLEN DEVELOPMENT AND FERTILITY

| Pedigree number | Chromosome number | Percentage of poor pollen | Fertility |
|-----------------|-------------------|---------------------------|-----------|
| A2-10 .....     | 10                | 1.9                       | Fair      |
| A2-15 .....     | 10                | 1.4                       | Poor      |
| B6-2 .....      | 10                | 2.9                       | Fair      |
| A2-15-5 .....   | 10                | 0.6                       | Good      |
| B6-2-5 .....    | 10                | 2.5                       | Fair      |
| A2-15-5-2 ..... | 10                | 1.3                       | Fair      |
| A2-10-1 .....   | 10                | 0.9                       | Good      |
| *A11-2-5 .....  | 10                | 4.2                       | Poor      |
| *A11-2-1 .....  | 10                | 4.5                       | Fair      |
| *A11-2-6 .....  | 10                | 2.1                       | Fair      |
| *B7-8-2 .....   | 10                | 0.7                       | Poor      |
| *A11-4-3 .....  | 10                | 18.7                      | Poor      |

## AVERAGE PERCENTAGE OF POOR POLLEN FOR 10-CHROMOSOME PLANTS, 3.4

|                  |    |      |      |
|------------------|----|------|------|
| A11-2 .....      | 11 | 14.5 | Poor |
| A11-2-8 .....    | 11 | 20.1 | Poor |
| *A11-4-4 .....   | 11 | 10.1 | Fair |
| *A11-4-5 .....   | 11 | 1.9  | Fair |
| *A2-10-1-1 ..... | 11 | 21.2 | ?    |

## AVERAGE PERCENTAGE OF POOR POLLEN FOR 11-CHROMOSOME PLANTS, 13.5

|                  |    |      |      |
|------------------|----|------|------|
| B7-3 .....       | 12 | 34.3 | Poor |
| A11-2-1 .....    | 12 | 22.1 | Fair |
| B7-8 .....       | 12 | 17.0 | Fair |
| *A11-2 .....     | 12 | 13.2 | Poor |
| *B7-1-5 .....    | 12 | 21.7 | Poor |
| *A11-2-8-7 ..... | 12 | 50.2 | Poor |
| *B7-1-3-1 .....  | 12 | 2.3  | ?    |
| A11-4-3-3 .....  | 12 | 10.9 | ?    |

## AVERAGE PERCENTAGE OF POOR POLLEN FOR 12-CHROMOSOME PLANTS, 21.4

|                  |    |      |      |
|------------------|----|------|------|
| B7-1 .....       | 13 | 27.2 | Poor |
| A11-4 .....      | 13 | 9.1  | Fair |
| *B7-3-9 .....    | 13 | 48.1 | Poor |
| *A11-2-1-1 ..... | 13 | 50.0 | Poor |

## AVERAGE PERCENTAGE OF POOR POLLEN FOR 13-CHROMOSOME PLANTS, 33.6

\* Either an individual plant or an average of two plants.

If the pollen grains containing supernumeraries are the nonfunctional ones, it is hard to see why the higher-numbered forms do not yield chiefly 10-chromosome plants. The explanation of the results obtained may lie in the possible occurrence of functional maternal gametes with more than five chromosomes or of nonfunctioning pollen grains having five chromosomes which lack something contained in the extra chromosome.

Little evidence of correlation between pollen development and fertility has been shown in the results obtained up to the present time. To be sure, the 10- and 11-chromosome plants show a higher degree of fertility and a lower percentage of deficient pollen in general. Among individual plants, however, those with the best pollen are not necessarily the most fertile. The 11-chromosome plants are virtually as fertile as those with ten, while 12's, 13's and 14's are nearly sterile and the rest completely so; and these do not show a proportional increase in nonfunctional pollen grains.

#### RESULTS OF CROSSES WITH *CREPIS ALPINA*

Following the method outlined by Hollingshead (1930), several crosses were made between individuals of *C. syriaca* from accession number 1923, and *C. alpina* plants obtained from accessions 2783, 1641, and 2769, in order to determine, as far as possible, cytogenetical relationships between the species.

When *C. syriaca* is used as the female parent there is a much higher percentage of seed set than in the reciprocal cross. This may be due to the fact that usually *syriaca* plants were chosen which had a chromosome number higher than ten; and it has been shown in wheat hybrids involving plants which differ in chromosome numbers, that it is preferable to use the plant with the higher number as female (Watkins, 1927). Only eleven of the achenes obtained from the cross *alpina* ♀ × *syriaca* ♂ produced plants and of these all but two died before reaching maturity. A glance at plate 13, figure 3, shows the total dissimilarity of these to the other hybrids and to the parents. The cause of this dissimilarity is as yet unknown. It is possible that environmental conditions may provide the true explanation, although occurrences of unlike reciprocal hybrids have been reported. Further crosses are planned in order to clear up this question.

From the reciprocal cross, twenty-six hybrids were obtained, being derived from *syriaca* plants having ten, eleven, twelve, and thirteen somatic chromosomes. In all of them hybrid vigor was marked. With respect to leaf type and general habit they were more like the *alpina* parent, but they had the flower-head type and color and the nodding buds of *syriaca*. Nearly all of them were virtually as early maturing as *syriaca* and were consistently highly fertile. The latter characteristic,

along with the compatibility of their chromosomes, is evidence of the close relationship of the two species.

In crosses where *alpina* was used as a female parent, the resulting hybrids all possessed ten chromosomes. This, of course, may only be because of the small number obtained. However, in view of the amount of variation ordinarily met with and the fact that only plants with supernumeraries were used as pollen parents, it is much more likely that there is a preferential functioning of pollen grains in favor of 5-chromosome grains.

In the *syriaca* ♀ × *alpina* ♂ crosses, the hybrids revealed varying numbers of chromosomes, the situation being similar to that in the female parent. The resulting chromosome numbers are shown in table 4. Since it may be assumed that the *alpina* parent always contributes five chromosomes, the number present in functioning egg-cells is readily calculated.

TABLE 4  
FREQUENCIES OF HYBRIDS HAVING DIFFERENT CHROMOSOME NUMBERS

| Parental chromosome numbers | 10 | 11 | 12  | 13  | Chromosome number in functioning egg-cells |
|-----------------------------|----|----|-----|-----|--|
| 10 × 10                     | 13 | .  | .   | .   | 5  |
| 11 × 10                     | 1  | 2  | ... | ... | 5, 6                                       |
| 12 × 10                     | 4  | 1  | 2   | 1   | 5, 6, 7, 8                                 |
| 13 × 10                     | .  | 2  | .   | .   | 6  |

As with *C. syriaca*, the  $F_1$  progeny of 10-chromosome parents all possessed ten chromosomes. Where a higher number was involved, variation again occurred and to a considerable degree. The somatic complements of the hybrids resemble closely the ones which have been described in connection with different number-groups of *syriaca*. The basic five pairs are consistently present with one, two, or three of the small supernumeraries, the appearance of which has already been described. Assuming a regular reduction division, we should expect that the progeny of a 12- and a 10-chromosome plant would contain eleven chromosomes. Such, however, does not prove to be true. Not only are both the extra chromosomes present in the offspring, but even an additional one may be added to yield a 13-chromosome form. As has already been indicated by the production of an 18-chromosome *syriaca* individual from a 13-chromosome parent, there must be a further multiplication of extra chromosomes in the divisions prior to, or during, meiosis. If feasible, a study of embryo-sac mother-cells should be of great assistance in clearing up this point. It has already been pointed out that *syriaca* pollen probably does not transmit the extra chromosome in crosses with *C. alpina*. Furthermore, the entire lack of resemblance of these individuals to their maternal parents



and their similarity to one another precludes the assumption of a development initiated by a doubling of the maternal genom. It is possible that *syriaca* pollen may transmit the extra chromosomes in self-fertilization, while showing quite a different behavior in *alpina* pistils.

### MEIOSIS IN THE $F_1$

An extensive investigation of meiotic behavior was not attempted since a preliminary survey indicated that this was not recognizably different from the parental type. This is only to be expected, considering the similarity between the chromosome groups of the two species involved and the high degree of fertility exhibited by the  $F_1$  plants. In all the 10-chromosome  $F_1$ 's examined, complete pairing was consistently the rule.

### POLLEN STUDIES IN THE $F_1$

Pollen counts of  $F_1$  plants revealed some rather unexpected results. It is significant, for example, that in 10-chromosome plants the percentage of poor pollen was much greater than in *C. syriaca* itself while the percentage of fertility was as high, if not higher. This indicates that there is not a very close correlation between percentage of good pollen and degree of fertility. However, there is always considerable variation even within an individual plant from time to time, so that these results are not conclusive evidence on this point. The percentages of poor pollen and the parental chromosome numbers of the  $F_1$  cultures are shown in table 5.

TABLE 5  
PARENTAL CHROMOSOME NUMBER AND POLLEN DEVELOPMENT IN  $F_1$

| Culture number | Parental chromosome number | Average percentage of poor pollen |
|----------------|----------------------------|-----------------------------------|
| X              | 10 x 10                    | 58.7                              |
| X3             | 10 x 10                    | 34.6                              |
| X8             | 10 x 10                    | 59.4                              |
| X2             | 11 x 10                    | 41.1                              |
| X4             | 12 x 10                    | 64.7                              |
| X6             | 13 x 10                    | 90.1                              |

The fact that in hybrids the percentage of undeveloped pollen was higher than in the parental species, irrespective of chromosome number, was of assistance in determining whether a hybrid had actually been obtained. Thus, a supposed hybrid which appeared very like *C. syriaca* in external morphology, was found to have only 1 per cent poor pollen and was for this reason classified as resulting from accidental self-fertilization.

The number of plants examined was of necessity very small, but, even so, it seems fair to draw some tentative conclusions from the examinations. The individual results are not tabulated because of the variation just referred to, but they indicate that the parental chromosome number had a greater influence on pollen production than the number actually contained in the individual. For example, two 11-chromosome plants in the progeny of a 13-chromosome *syriaca* parent had values of 89.1 per cent and 91.2 per cent poor pollen, while similar plants in the progeny of an 11-chromosome progenitor had only about 40 per cent of undeveloped grains.

As before, there is an increase in proportion of nonfunctioning grains with an increase in number of extra chromosomes. In spite of the unbalance in the 11-chromosome plants, they produce progeny which exhibit considerably less poor pollen than the 12's or 13's. This is especially surprising in view of the fact that six pairs are consistently found in 12-chromosome plants.

### F<sub>2</sub> CHROMOSOME NUMBERS

From the results discussed so far it might reasonably be expected that in the F<sub>2</sub> progeny a variability in chromosome number similar to that in the parental and F<sub>1</sub> types would be expressed. In respect to the 10-chromosome lines this proved to be true, all individuals determined hav-

TABLE 6  
FREQUENCIES OF F<sub>2</sub> PLANTS WITH DIFFERENT CHROMOSOME NUMBERS

| Culture number | Parental number | Frequencies of plants with: |     |     |     |    |    |
|----------------|-----------------|-----------------------------|-----|-----|-----|----|----|
|                |                 | 10                          | 11  | 12  | 13  | 14 | 15 |
| X3-2           | 10              | 10                          | ... | ... | ... | .  | .  |
| X4-1           | 10              | 9                           | ... | ... | ... | .  | .  |
| X2-2           | 11              | .                           | 1   | 9   | ... | .  | .  |
| X6-1           | 11              | 1                           | .   | 8   | .   | .. | .  |
| X7-1           | 12              | .                           | .   | 1   | 1   | 2  | 1  |

ing ten somatic chromosomes. However, in the progeny of 11-chromosome F<sub>1</sub> individuals the majority of plants had twelve, while the self-pollination of a 12-chromosome type gave rise to considerable variation again, the tendency being toward still higher numbers. The obtaining of such a large proportion of 12-chromosome plants from an 11-chromosome parent is very difficult to explain. Referring to the 11-chromosome groups of tables 1 and 2, it can be seen that eighteen out of a total of twenty-eight had eleven chromosomes, while only three had twelve. In these F<sub>2</sub> cultures seventeen out of a total of nineteen had twelve, while only one had eleven (cf. table 6). This may indicate a tendency to pro-

duce a balanced number of chromosomes and suggests again the theory that the supernumeraries contain some material which is beneficial in development. When achenes from 12-chromosome  $F_1$  plants were sown, however, the resulting plants, which are obtained only with difficulty, showed greater variation in chromosome numbers than the progeny of 11-chromosome individuals, in spite of the unbalance in the cells of the latter type.  $F_2$  results should prove of great interest in connection with the stability of different chromosome groups. In table 6 are shown the frequencies of  $F_2$  plants having various chromosome counts. These plants were all quite uniform and, as in  $F_1$ , they resembled their *alpina* grandparents in habit, leaf type, and fertility, and showed segregation with respect to flower-head type. They did not mature quite so early as the  $F_1$ , but much earlier than pure *alpina*.

## DISCUSSION

Supernumerary chromosomes have been reported in *Zea* (Randolph, 1928), *Secale* (Gotoh, 1924; Belling, 1925), *Ranunculus* (Langlet, 1927; Sorokin, 1927), *Oenothera* (Lutz, 1916), *Rosa* (Blackburn and Harrison, 1921), *Crepis* (Navashin, 1926), *Fritillaria* (Darlington, 1930), *Viola* (Clausen, 1931), and in a number of insects (Stevens, 1908; Carothers, 1917; Wilson, 1909). In most of these the extra chromosomes arise by (1) *duplication*, (2) *fragmentation*, or (3) *hybridization*.

*Duplication* of chromosomes by means of nondisjunction or nonconjunction was found by Navashin to be the cause of a small amount of spontaneous chromosomal alteration in *Crepis tectorum*. The supernumeraries in this species had a greater effect on the viability of the plants than those found in *C. syriaca*. The morphology of the extra unit in the latter species precludes the possibility of its origin by duplication although this may be a factor in the multiplication of it in higher-numbered plants.

*Fragmentation* of chromosomes of the normal complement is assumed to be the cause of the extra units in *Secale*, *Fritillaria*, and *Oenothera*. According to Geerts (1911) this phenomenon is likely to occur in extra chromosomes as a result of hybridization. Measurements of chromosome lengths in *C. syriaca* revealed that there was a definite linear increase of the chromosomes as a group where supernumeraries were present. Thus it is very unlikely that they arose by a simple fission of a member of the basic genom. It is possible that fragmentation following an attachment of two chromosomes may have led to the production of the X chromosomes of *C. syriaca*. Carothers (1917) explains in this way the origin of extra units in *Trimerotropis* and *Circotettix*.

*Hybridization* is the factor to which is ascribed the origin of extra chromosomes in *Ranunculus* and *Viola*. The fact that in *C. syriaca* varia-

bility is so widespread suggests that the species has arisen by hybridization (involving *C. alpina*, in all probability). So far, no material has been examined which did not show a fairly high proportion of individuals with extra chromosomes, although in all the material the chromosome number was constant for the individual. Furthermore, there seems to be no tendency toward reversion to the 10-chromosome type. Plants of this type are regularly found to be in the majority and, when selfed, yield only 10-chromosome progeny, yet accessions from the wild which were studied showed many extra-chromosome individuals.

The only related species which possesses a chromosome similar to the extra units found in *C. syriaca* is *C. rubra*. This species is indigenous in Greece and is not known from Syria, the home of *syriaca*, nor even from Asia Minor. If natural hybridization with *C. rubra* has brought about the increase in chromosome number, it must be assumed that the cross was effected a very long time ago and that the unbalanced condition has been maintained. Furthermore, a careful examination of external morphology fails to reveal the presence of any distinctive *rubra* characters in *syriaca*. For these reasons it is necessary to look further for the origin of the supernumeraries.

Although it appears unlikely that *C. rubra* was involved with *C. alpina* in the origin of *C. syriaca*, because of their wide geographic separation and the absence of any distinctive *rubra* characteristics in *syriaca* plants, there are other possible explanations involving natural hybridization. It has already been pointed out that two distinct strains of *C. alpina* are known which grow in fairly close proximity to each other, the typical form being distributed from Asia Minor to Transcaucasia, the other in the Caucasus region. Furthermore, the latter type possesses some characters which are more like those of *syriaca* than those of the typical form. It is quite possible that, after the former became distinct, natural crossing was effected between them, which, with later genic and chromosomal modifications, produced individuals of the *syriaca* type. These may have become isolated and gradually moved in a southerly direction to occupy the Lebanon region, while the typical form of *C. alpina* was restricted to the present more northern range and the other to the Caucasus and neighboring localities. This would explain the great similarity of the basic chromosome groups of all three races, as well as the origin of certain taxonomic characters of *C. syriaca*. For example, the flowers of the latter are brighter yellow than those of typical *C. alpina*, but are very similar in color to those of *alpina* plants from the Caucasus region. In habit and achene characters, also, *syriaca* and the Caucasian form of *alpina* show a tendency toward mutual resemblance. Such a hybridization might lead to an alteration in the normal chromosome behavior, giving rise to the variation characteristic of *C. syriaca*.

Another explanation of the origin of these forms is that *C. syriaca* and *C. alpina* (Caucasian type) are both the result of hybridization with some form now unknown. This is suggested by the fact that the Caucasian form is more or less intermediate between *C. syriaca* and typical *C. alpina*. This interpretation, however, is less satisfactory since it assumes the existence of an unknown form. Moreover, the present situation in respect to both comparative morphology and the chromosomes in the two forms of *C. alpina* and *syriaca* can be reasonably explained without recourse to this alternative hypothesis.

Navashin (1931a) has shown how chromosomes may become altered in nature. Plants of *C. tectorum* which were abnormal in appearance showed, upon cytological examination, that chromosomes quite unlike those among the normal four pairs were present. These had arisen by a fragmentation of the normal members, followed by attachments of the fragments so produced. He points out that Dobzhansky (1930) has found such alterations occurring in homozygous condition. The individuals containing them were viable and even capable of reproducing themselves.

A preliminary study of plants grown from accession number 3106 indicates that a similar situation exists in *C. syriaca*. In these individuals many types of chromosomal abnormality are to be found, including such units, appearing as supernumeraries, as are found in the cultures derived from accession number 1923. Let us assume that hybridization of the two forms of *C. alpina* has produced *syriaca*-like progeny. Either as a result of this or through some environmental agency (temperature, chemical condition of the soil, natural radiation, etc.), an alteration in chromosome affinity is brought about. Two nonhomologous chromosomes become attached in the prophase stages of cell division and, upon separation, do not retain their original form. For example, a part of one of the other chromosomes might remain attached to the satellite of the normal D chromosome. A similar effect would be produced if fragmentation preceded the attachment. Where fragments are produced which do not possess a spindle-fiber attachment, they are subsequently lost unless they become attached to a unit which possesses one. Such an attachment of a fragment would produce a chromosome with an abnormally large satellite, as in the X chromosome described earlier. If, in reality, the supernumerary has arisen by means of a modification of the normal D chromosome, this alteration would also necessarily involve a shortening of the distal arm by fragmentation or some other means. It is altogether likely that an alteration of this kind would upset the normal chromosome balance to such an extent that sterility would result. In *syriaca*, however, the X chromosome seems to have only a weak effect when present one, two, or even three times. Thus, this particular chromosome may have been introduced into otherwise normal complements and

been able to reproduce itself even in competition with other more normal types.

The fact that the extra chromosomes are reproduced in such a constant manner lends strength to the theory of chromosome individuality. Other similar examples have been used as reason for questioning its validity.

Little work has been done, to the writer's knowledge, with respect to studies of progeny from extra-chromosome plants of this type. The present study shows the tendency of certain of the higher-numbered individuals to maintain these complements. It is probable that, by continuing the work, stable races could be established.

The investigation of cytogenetic relationships of *C. alpina* and *C. syriaca* is of interest in the light of modern theories in taxonomical research. From a purely morphological consideration there is little doubt that they should be classified as distinct species. Constant differences are apparent wherever material is found, and although they do exist in the same general geographical region, their distribution is not known to overlap. Certain intermediate characters indicate that *syriaca* has arisen from *alpina* originally, but changes have occurred which make its segregation warranted.

The criteria which, cytogenetically speaking, have so far been of assistance in taxonomic treatments, are: (1) number of chromosomes, (2) chromosome size and shape, (3) compatibility of chromosomes when brought together in a hybrid, and (4) interfertility or sterility upon hybridization. As has been pointed out, the chromosomes of the two species under discussion are alike in both number and morphology with the exception of the supernumeraries present in *C. syriaca*. When crossed, a hybrid is obtained which is more or less intermediate in appearance. It shows a high degree of chromosome affinity and is as fertile as the parental species, or nearly so. Thus, with reference to chromosome affinity in the basic genom and the fertility of  $F_1$  hybrids, the former classification, that is, treating *syriaca* as a subspecies of *C. alpina*, is probably justified. There remain, however, the supernumerary chromosomes which are unique in *syriaca* and the numerous differences in external morphology which serve to set it apart as a species.

## SUMMARY

1. *Crepis syriaca* was originally described as a variety of *C. alpina* L., and unquestionably it is very closely related, but numerous constant morphological differences exist.

2. Accessions from the wild show considerable chromosomal variation involving number and morphology. Chromosome numbers are constant for the individual, but in selfed lines plants with ten, eleven, twelve,

thirteen, fourteen, fifteen, sixteen, and eighteen somatic units are found, the variation involving a small chromosome of distinctive morphology. Selfed cultures show a much greater stability of 10-chromosome lines and greater variability of lines derived from plants containing supernumeraries. This variability does not lead to the production of a majority of 10-chromosome individuals.

3. The supernumeraries have small and inconsistent effects on the external morphology of plants containing them. Abnormal flower-head development is regularly associated with the presence of two or more extra units. Ten- and 11-chromosome plants were very similar in appearance, but higher-numbered individuals varied considerably from the normal. An interesting new "dwarf" form, not associated with chromosome variation, made its appearance in 10-chromosome cultures.

4. The presence of extra chromosomes causes a marked increase in the percentage of undeveloped pollen grains produced, this increase being more or less proportional to the number of extra units. The number of supernumeraries present apparently has a greater effect in this connection than has the chromosome unbalance. There seems to be a preferential functioning in favor of gametes with more than five univalents in plants with more than ten chromosomes. Although individuals with five or more supernumeraries are entirely sterile and those with fewer are partly so, there does not seem to be a direct correlation between pollen development and fertility in these cultures.

5. At meiosis the basic genom forms five bivalents. In plants containing two or four supernumeraries complete pairing is consistently found. In 11-chromosome plants, the univalent divides at the first division. At the second, the halves go to one or the other pole and are incorporated in the daughter nuclei. Occasional nondisjunction of supernumerary units at these divisions may account for the increase in chromosome number.

6. In  $F_1$  hybrids from *C. syriaca*  $\times$  *C. alpina* the chromosome situation is closely similar to that in the pure species. So far, the extra chromosomes have been shown to be transmitted only by maternal gametes. The hybrids show complete pairing of chromosomes in 10-chromosome crosses and are highly fertile.

7. Although high fertility is shown by  $F_1$  individuals, the percentage of undeveloped pollen increases considerably, even in 10-chromosome plants. The parental genom apparently has a greater effect on the pollen development than has that of the progeny plant. The proportion of undeveloped pollen increases with the number of extra chromosomes involved in the cross.

8. *C. rubra*, a related species, has a chromosome resembling the supernumerary of *C. syriaca*; however, the possibility that it has been involved in the origin of the latter species by hybridization is rejected on grounds of evidence from comparative morphology and geographic distribution.

9. A more acceptable hypothesis assumes the origin of *C. syriaca* through hybridization between typical *C. alpina* and a Caucasian form of *alpina* followed by chromosomal alterations in some of the hybrids, giving rise to the supernumerary chromosomes which, in this species, are unique.

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**Plate 12**

***Crepis syriaca***

- Fig. 1.** Dwarf strain (ten chromosomes).  
**Fig. 2.** Normal plant (ten chromosomes).  
**Fig. 3.** 11-chromosome plant.  
**Fig. 4.** 12-chromosome plant.



*Fig. 1*



*Fig. 2*



*Fig. 3*



*Fig. 4*

Plate 13

*Crepis syriaca*

Fig. 1. 14-chromosome plant.

*Crepis alpina* and F<sub>1</sub> hybrids with *C. syriaca*

Fig. 2. *C. alpina* (ten chromosomes).

Fig. 3. 10-chromosome hybrid from cross *C. alpina* ♀ x *C. syriaca* ♂.

Fig. 4. 10-chromosome hybrid from cross *C. syriaca* ♀ x *C. alpina* ♂.



*Fig. 1*



*Fig. 2*



*Fig. 3*



*Fig. 4*

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### INTRODUCTION

THE FIRST PAPER of this series (Hollingshead and Babcock, 1930), presented data on the number and morphology of the chromosomes in sixty-seven species of *Crepis* and three other species belonging in closely related genera. Since then it has been possible to study the chromosomes of fifty additional species and subspecies, and it is now possible to discuss the bearing of all these chromosome studies on the phylogenetic relationships of about half the species in the genus.

Our conception of the fundamental principles of biological classification remains essentially as set forth in the contribution cited. More recently one of us (Babcock, 1931) has discussed the species-concept and emphasized the value of chromosome number and morphology as one criterion of classification according to natural relationship. In *Crepis* this criterion has proved especially valuable. The genus is large and much diversified, many species are rare or little known, and from comparative morphology of the plants alone it is often difficult to draw definite conclusions. From a comparative study of the chromosomes it is sometimes possible to obtain the first clue concerning the manner of origin of certain species, and such clues have led to the discovery of confirmatory evidence from other criteria.

### CYTOLOGICAL MATERIAL AND METHODS

Chromosomes are notoriously variable in appearance, even within a single individual, according to the conditions under which they are studied. Due precautions have therefore been observed for the study of comparable material. As in our earlier work and in most of the other researches in this field, the appearance of the chromosomes at mitotic metaphase has been used. Considerable refinement in the technique of comparing size and shape of the chromosomes has been attained by making camera lucida drawings of the best available diploid groups, identifying the several pairs present in the group, and deriving therefrom the members of the haploid genom. The illustrations in the present paper depict these haploid genoms all drawn to the same scale.

TABLE 1  
CHROMOSOME NUMBERS IN FIFTY-FIVE SPECIES, SUBSPECIES, AND FORMS NOT  
PREVIOUSLY REPORTED

| CREPIS—OLD WORLD                                     |                                 |                                 |                             |          |
|--|---------------------------------|---------------------------------|-----------------------------|----------|
| Species  | Somatic<br>chromosome<br>number | Number<br>of plants<br>examined | Accession                   | Subgenus |
| * <i>C. albida asturica</i> (Lacaita<br>et Pau)..... | 10                              | 3                               | 2088.....                   | B        |
| <i>C. albida macrocephala</i><br>(Willk.).....       | 10                              | 2                               | 2957.....                   | B        |
| <i>C. alpestris</i> (Jacq.) Tausch.....              | 8                               | 2                               | 2512.....                   | C        |
| <i>C. argolica</i> Babc.....                         | 8                               | 2                               | 2884.....                   | E        |
| <i>C. argolica tirynica</i> Babc.....                | 8                               | 3                               | 3036.....                   | E        |
| <i>C. aspera jordanensis</i> Babc.....               | 8                               | 3                               | 3010.....                   | B        |
| <i>C. aurea lucida</i> (Ten.).....                   | 10                              | 2                               | 2912.....                   | C        |
| <i>C. bellidifolia</i> Loisel.....                   | 8                               | 1                               | 2921.....                   | B        |
| <i>C. bhotanica</i> Hutchinson.....                  | 16                              | 2                               | 3216, 3245.....             | C        |
| <i>C. bifida</i> (Vis.) F. et M.....                 | 10                              | 6                               | 3057, 3083, 3084, 3087..... | E        |
| <i>C. bithynica</i> Boiss.....                       | 10                              | 3                               | 3218.....                   | E        |
| <i>C. canariensis</i> (Sch. Bip.).....               | 8                               | 3                               | 3049.....                   | B        |
| <i>C. clausonis</i> Pomel.....                       | 8                               | 4                               | 2848.....                   | B        |
| † <i>C. crocea</i> (Lam.).....                       | 16                              | 6                               | 2174, 2352, 2353.....       | C        |
| <i>C. divaricata</i> (Lowe) F.<br>Schultz.....       | 8                               | 4                               | 2980.....                   | B        |
| <i>C. eigiana</i> Babc.....                          | 8                               | 4                               | 3125, 3138.....             | E        |
| <i>C. eritreënsis</i> Babc.....                      | 10                              | 1                               | 3005.....                   | B        |
| <i>C. flexuosa</i> (DC.) Benth. et<br>Hook. f.....   | 14                              | 2                               | 2983.....                   | E        |
| <i>C. fontiana</i> Babc.....                         | 8                               | 3                               | 3225.....                   | B        |
| <i>C. fuliginosa</i> S. et S.....                    | 6                               | 11                              | 2994, 3043, 2891.....       | E        |
| <i>C. granatensis</i> Babc.....                      | 8                               | 4                               | 1894.....                   | E        |
| † <i>C. hieracioides</i> Lowe.....                   | 8                               | 5                               | 2817, 2818, 2823.....       | B        |
| <i>C. hyemalis</i> (Biv.) C. P. et<br>G.....         | 8                               | 3                               | 3053.....                   | B        |
| <i>C. hypochaeridea rhodesica</i><br>Babc.....       | 8                               | 1                               | 3059.....                   | C        |
| ¶ <i>C. juvenalis</i> (Delile) F. Sch.....           | 8                               | 7                               | 3205, 3206, 3207.....       | B        |
| <i>C. kashmirica</i> Babc.....                       | 12                              | 4                               | 3186.....                   | C        |
| <i>C. multicaulis congesta</i><br>(Rgl.).....        | 10                              | 3                               | 3187.....                   | E        |
| <i>C. mungieri</i> Boiss.....                        | 12                              | 9                               | 2870, 2876, 2877.....       | E        |
| <i>C. myriocephala</i> Coss. et<br>DR. 4n forms..... | 16                              | 6                               | 2844, 2845.....             | B        |
| <i>C. nigricans</i> Viv.....                         | 8                               | 3                               | 3020.....                   | B        |
| <i>C. oreades</i> Schrenk.....                       | 8                               | 1                               | 2981.....                   | E        |

\* Cf. *C. asturica* (Hollingshead and Babcock, 1930).

† Cf. *C. bungei* 2174 (Hollingshead and Babcock, 1930).

‡ One triploid plant.

¶ One trisomic.

TABLE 1—(Concluded)

| Species                                       | Somatic chromosome number | Number of plants examined | Accession   | Subgenus |
|---|---------------------------|---------------------------|---|----------|
| <i>C. patula</i> Poiret.....                  | 8                         | 2                         | 2839.....   | E        |
| <i>C. polytricha</i> Turcz.....               | 16                        | 3                         | 2562.....   | C        |
| <i>C. pterothecoides</i> Boiss.....           | 8                         | 3                         | 3232.....   | E        |
| <i>C. pumila</i> (Lowe).....                  | 8                         | 2                         | 3022.....   | B        |
| <i>C. pygmaea</i> L.....                      | 12                        | 1                         | 3251.....   | E        |
| <i>C. raulinii</i> Boiss.....                 | 10                        | 3                         | 2875.....   | E        |
| <i>C. reuteriana</i> fa. <i>hirta</i> Babco.  | 8                         | 2                         | 3134.....   | E        |
| <i>C. robertioides</i> Boiss.....             | 8                         | 2                         | 3129.....   | E        |
| <i>C. sancta beirutica</i> Babco.....         | 10                        | 3                         | 3160.....   | E        |
| <i>C. setosa topaliana</i> Babco.....         | 8                         | 3                         | 2906.....   | B        |
| <i>C. stojanovii</i> T. Georg.....            | 8                         | 2                         | 3176.....   | E        |
| <i>C. suberostris</i> Batt.....               | 10                        | 1                         | 2829.....   | B        |
| <i>C. suffreniana</i> (DC.) Lloyd             | 8                         | 3                         | 2975.....   | B        |
| <i>C. tarazacifolia laciniata</i> (Lowe)..... | 8                         | 3                         | 2803, 2815, 2819..                                  | B        |
| <i>C. tarzacoides</i> Desf.....               | 16                        | 2                         | 2944.....   | B        |
| <i>C. taygetica</i> Babco.....                | 40                        | 3                         | 2893.....   | E        |
| <i>C. thomsonii</i> Babco.....                | 10                        | 1                         | 3208.....   | B        |
| <i>C. triasii</i> (Camb.) Fries.....          | 8                         | 2                         | 2945, 2949.....                                     | B        |
| <i>C. tubaeformis</i> Halacsy.....            | 8                         | 3                         | 3066, 3069.....                                     | E        |
| <i>C. vesicaria</i> L. 4n forms.....          | 16                        | 3                         | { 2851, 2852, 2853, 2854,<br>2947, 2948, 3056, 3203 | B        |
| <i>C. viscidula</i> Froel.....                | 12                        | 3                         | 3178.....   | C        |
| <i>C. willemetioides</i> Boiss.....           | 12                        | 3                         | 3217.....   | E        |

## CREPIS—AMERICAN

|                                       |     |   |           |   |
|---------------------------------------|-----|---|-----------|---|
| <i>C. atribarba</i> A. A. Heller..... | 88? | 2 | 3045..... | E |
|---------------------------------------|-----|---|-----------|---|

## OTHER GENERA

|  |    |   |           |  |
|--|----|---|-----------|--|
| <i>Lactuca depressa</i> (Hook. f. et Thom.) ( <i>Crepis depressa</i> )               | 16 | 1 | 3246..... |  |
| <i>Prenanthes glomerata</i> Dcne. ( <i>Crepis glomerata</i> Benth. et Hook. f.)..... | 16 | 1 | 3252..... |  |

|| Cf. *C. polytricha* (Babcock and Navashin, 1930).

Root-tips for this study were fixed in chrom-acetic-formalin solution 1, as described on page 3, Hollingshead and Babcock (1930). Paraffin sections were cut from 8 to 12 $\mu$  thick and stained either in Heidenhain's iron haematoxylin or crystal violet. A Zeiss 1.3 oil immersion objective and Zeiss compensating ocular were used throughout this study. Drawings were made with a camera lucida at a magnification of 3750 and reduced to 2500 in reproduction. In all other respects a procedure was adopted which would produce results comparable with those reported by Hollingshead and Babcock (1930).

## CHROMOSOME NUMBERS

The chromosome numbers of sixty-eight species of *Crepis* have already been reported. In table 1 are given the diploid numbers of forty species, nine subspecies, and several forms not previously reported. One subspecies is relisted because of a change in nomenclature; and one species (*polytricha*) appears here because it was not reported in the first paper of this series. The species commonly known as *Crepis glomerata* was excluded from *Crepis* by Babcock and Navashin (1930). This species has been referred to *Prenanthes*, where it was originally classified by its author. Another species, long known as *Crepis depressa*, has been referred to *Lactuca*. These two excluded species are listed at the end of table 1 for purposes of record. Classification of the *Crepis* species according to subgenus is shown in table 1, right-hand column; C = *Catonia*, E = *Eucrepis*, B = *Barkhausia*.

## CHROMOSOME NUMBER AND PHYLOGENY

### THE SUBGENERA

In earlier publications four subgenera have been recognized, namely, *Paleya*, *Catonia*, *Eucrepis*, and *Barkhausia*. More recent studies show that the four species previously classified in *Paleya* are primitive representatives of *Catonia* and *Barkhausia*, therefore the subgenus *Paleya* has been merged with the two just mentioned. This makes possible a more satisfactory representation of phylogenetic relations.

In order to discuss the bearing of chromosome number on phylogeny, it is necessary first to consider the relationships of the three subgenera as determined from other evidence. It is not proposed to present all this evidence in detail here but merely to indicate the general situation as clearly as possible. In addition to the number of species in each subgenus, their duration of life (whether perennials or annuals and biennials), and their geographic distribution, certain aspects of their comparative morphology have been found especially valuable. The morphological characters used in differentiating the subgenera are presented most readily in the form of an analytical key.

### KEY TO THE SUBGENERA

- Bracts of the mature involucre unchanged or merely indurate.....*Catonia*  
 Bracts of the mature involucre dorsally keeled or spongy-thickened  
   or both.  
   Achenes unbeaked or only very shortly or coarsely beaked (rarely  
     with beak equal to body).....*Eucrepis*  
   Achenes definitely beaked; the beak usually long and slender.....*Barkhausia*

Without going into details or pausing to discuss certain exceptional species which are difficult to classify according to the foregoing scheme, it is obvious that there is progressive specialization in the structure of both involucre bracts and achenes. Along with this increasing specialization there is a definite trend toward reduction in length of life. Thus, all *Catonia* species are perennial; while one-fourth of the *Eucrepis* and three-fourths of the *Barkhausia* species are annual. The evidence from

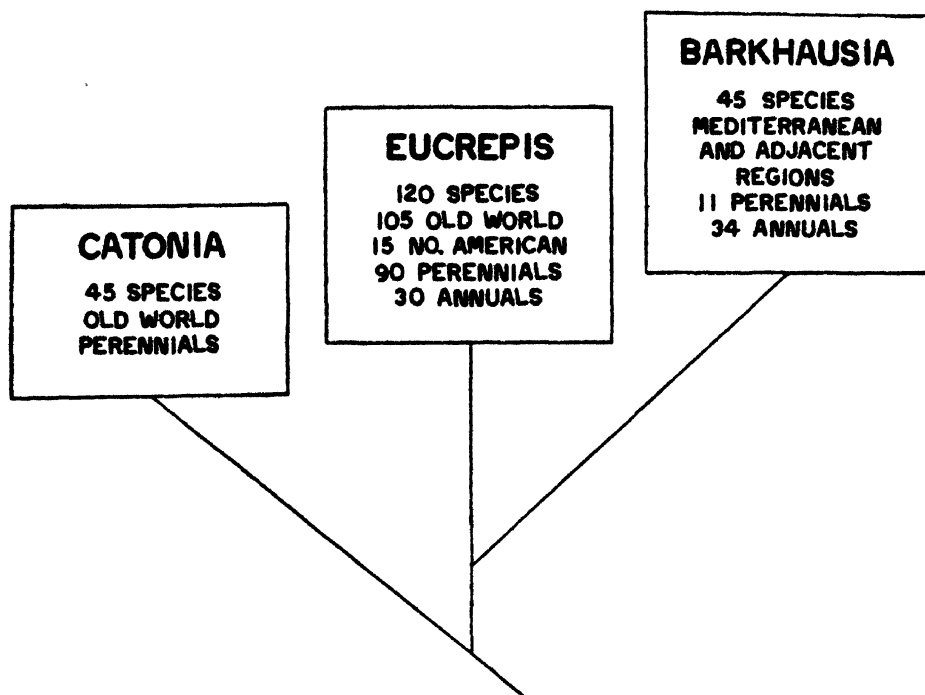


Fig. 1. Composition and phylogenetic status of the subgenera of *Crepis*.

geographic distribution will not be presented here; in general it is in agreement with the inference that *Catonia* is the most primitive, and *Barkhausia* the most recent group, while *Eucrepis* is intermediate. Furthermore, there are a number of border-line species in *Eucrepis*, some of which verge toward *Catonia* and others toward *Barkhausia*. Thus, the general situation in respect to phylogenetic relations between the subgenera may be represented as in figure 1.

*Chromosome numbers in the subgenera.*—The distribution of chromosome numbers in the three subgenera is shown in figure 2. An exponent indicates the number of species having a given chromosome number. This representation reveals several significant facts. It will be noted that the entire series of chromosome numbers is found in *Eucrepis*, while *Catonia* and *Barkhausia* have comparatively small series. But in each subgenus, as here represented, there is more than one basic number.

The basic numbers common to all three subgenera are 8 and 10, there being fifty-five species with eight, and nineteen species with ten chromosomes. In *Catonia* and *Eucrepis* there is a third basic number, namely, 12, and in *Eucrepis*, a fourth, namely, 14. But as will be shown, these "basic" numbers are not all equally primitive. Furthermore, the subgenera may contain more than one phylogenetic line of a given basic number.

## CHROMOSOME NUMBER AND PHYLOGENY IN CREPIS

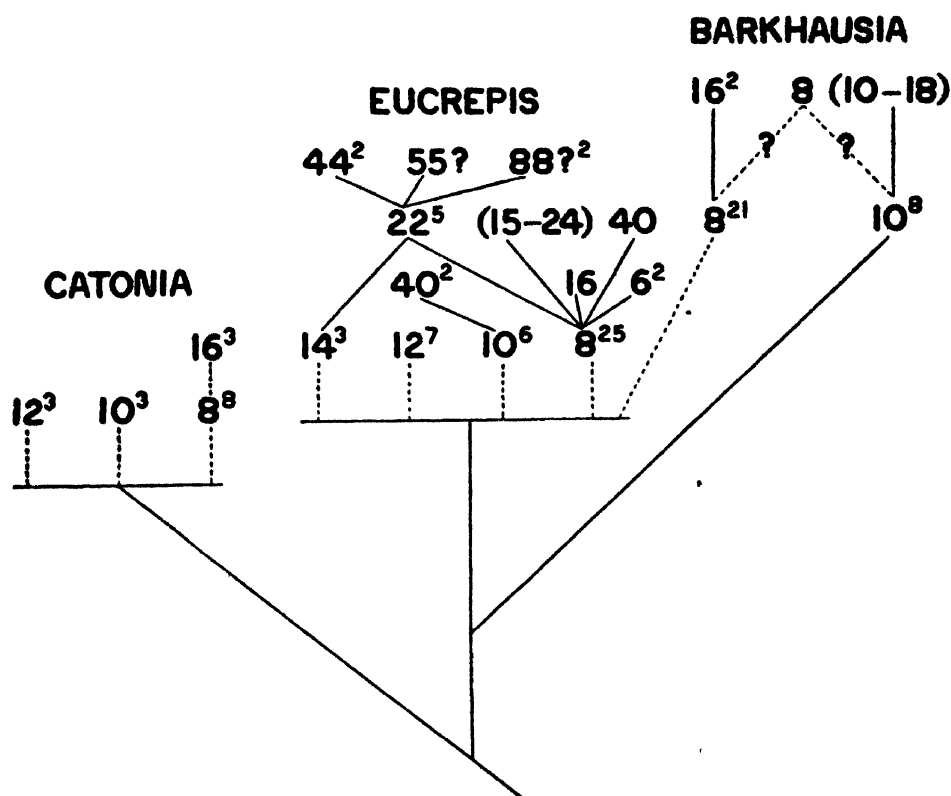


Fig. 2. Distribution of chromosome numbers in the subgenera in relation to phylogeny.

The great predominance of 8-chromosome species has led to the erroneous assumption by some writers that eight is the most primitive number in *Crepis*. But frequency of occurrence is not a sufficient basis for such an inference. The important fact that some of the 8-chromosome species are more highly specialized than any 10-chromosome species has been overlooked. Of still greater significance is the fact that none of the 8-chromosome species, even in *Catonia*, are as primitive in morphological aspects as are some of the 10-chromosome species, such as *sibirica* and *pontana* in *Catonia*, *raulini* and *bithynica* in *Eucrepis*, and *albida*

in *Barkhausia*. The 12- and 14-chromosome species present special problems which will be considered later. As between 8 and 10 the latter must be considered the more primitive number in *Crepis* or the progenitors of *Crepis*; but in the present representation 8, 10, 12, and 14 are all treated as basic numbers.

The diagram (fig. 2) also shows the comparative amount of differentiation in chromosome numbers in the three subgenera. In *Catonia* all the species have basic numbers except three, two of which certainly, and the third (*bhotanica*) probably, were derived from 8-chromosome ancestors. Predominance of basic chromosome numbers in this subgenus is associated with persistence of primitive morphological features. In *Barkhausia*, however, we have the most highly specialized portion of the genus. Yet all but three of the species thus far studied have the basic number 8 or 10. This immediately suggests the inference that the higher degree of specialization which is characteristic of *Barkhausia* has developed chiefly through other evolutionary processes than those involving changes in chromosome number. One such process, as has already been pointed out (Babcock and Navashin, 1930), is factor or point mutation (genovariation).

The greatest diversity in chromosome numbers occurs in *Eucrepis* and several different processes of alteration in chromosome number have been involved. From species with eight chromosomes there have been derived species with six by loss of one pair; species with sixteen by autotetraploidy; a species with fifteen, twenty, and twenty-four which seems to have arisen through amphidiploidy; polyploids with about forty. Species with eight and fourteen chromosomes respectively are believed to have hybridized and produced amphidiploids with twenty-two; and a polyploid series has been derived from the last. At the same time there are various degrees of specialization which may have been made possible largely by gene mutations.

#### CATONIA

The phylogenetic relations of seventeen species of *Catonia*, as determined primarily from gross morphology, geographic distribution and chromosome number, are shown in figure 3. It must be admitted that the evidence from chromosome morphology, to be presented below, has also been considered in arranging this diagram; also the indications of relationship to be found in natural and artificial hybrids between species. In *C. blattarioides*, for example, gross morphology alone seems to connect it more closely with *sibirica* and *pontana* than with *alpestris* and *hypochaeridea*, but the evidence from chromosome number and morphology and the occurrence of natural hybrids between *blattarioides* and *alpestris*, seem to outweigh the evidence from superficial appearance. In

general, however, the degree of morphological resemblance is roughly indicated by this diagram.

The 5-paired species, especially *sibirica* and *pontana*, are in several respects among the most primitive morphological types in the entire genus, while *aurea*, particularly subsp. *lucida*, exhibits the greatest re-

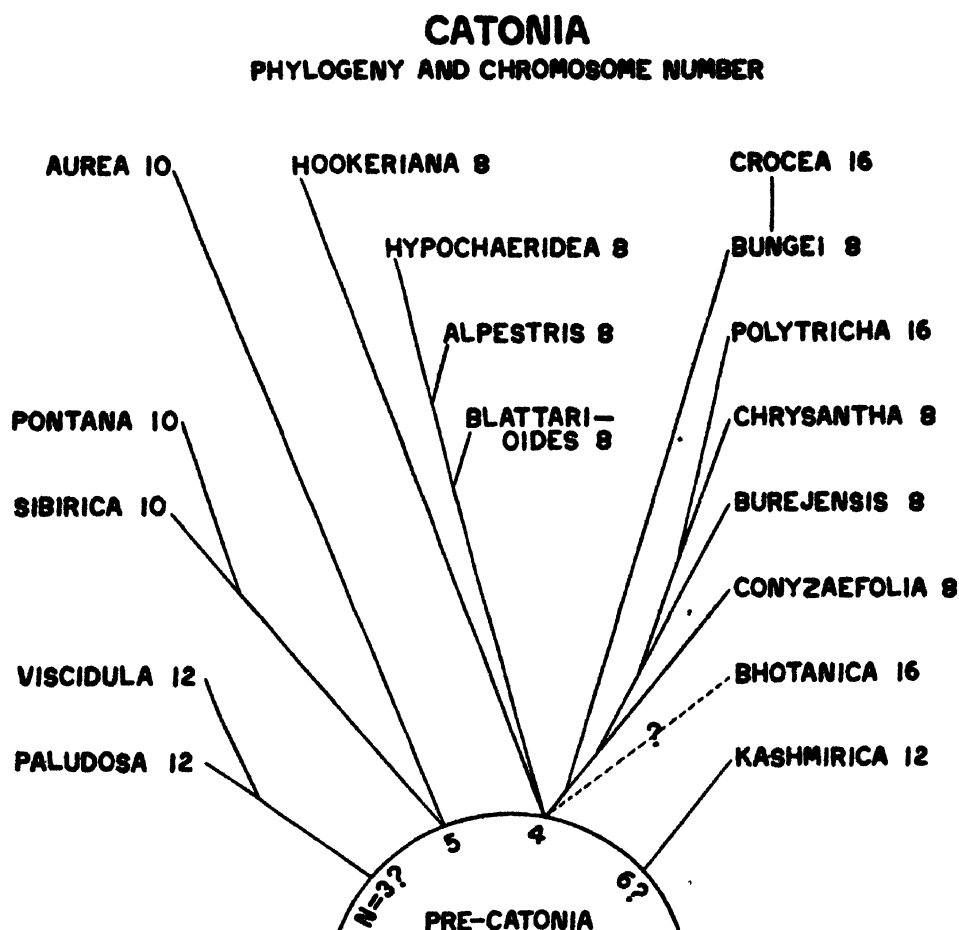


Fig. 3. Phylogenetic relations and chromosome numbers of seventeen species in subgenus *Catonia*.

duction in size of the whole plant and all its parts to be found in the *Catonia* species thus far studied cytologically.

The 4-paired *Catonia* species fall into two main groups and they may represent two or three different progenial stocks. On the left are shown the three species already mentioned and *C. hookeriana*, which is sufficiently like *hypochaeridea* to suggest that it diverged from the same stock. On the right are six species, *conyzaefolia-crocea*, which are evidently related and which may have sprung from the same stock as the 4-paired species on the left. The evidence for derivation of *crocea* from



*bungei* will be given below. Both *crocea* and *polytricha* are certainly polyploids with  $n = 4$  as the base number. *C. bhotanica* is a prominent species with sixteen chromosomes, which shows sufficient resemblance to those just above it in the diagram to warrant the assumption that it arose from the same 4-paired ancestral line; but our study of chromosome morphology has not gone far enough to demonstrate conclusively that it is a polyploid with  $n = 4$  as the base number.

The 12-chromosome *Catonia* species certainly represent two widely divergent lines, which differ greatly from each other morphologically as well as from all other species in the genus. On the extreme left are *paludosa* and *viscidula* which, in habit and fruit characters, show some resemblance to *Hieracium* species. Apparently they represent a rather stable primitive stock, because few if any other distinct species are known to belong in this group, although *paludosa* is one of the most widely distributed species in the genus. On the extreme right is *kashmirica*, which has long been confused with *blattarioides* although the resemblance is merely superficial. There is still some question whether these three 12-chromosome species are diploids or polyploids of some sort. The haploid number may be 3 or 6.

#### EUCREPIS

Fifty-nine species of *Eucrepis* are arranged in figure 4 according to phylogeny and chromosome number. Here again chromosome morphology has been considered together with other available criteria of relationship; and the degree of resemblance in gross morphology is roughly indicated by the arrangement of groups and within groups. In general the more primitive species are below and the more specialized forms above, with the exception of the two perennials, *oreades* and *robertioides*, which appear above *biennis* and *ciliata*, and the ten American species which are placed at the top because they are probably of comparatively recent origin.

The species having 4 as the haploid number are all on the left side of the diagram except the *oreades-suffreniana* assemblage on the upper right. Of all the 8-chromosome *Eucrepis* species, *patula* is in certain respects the most primitive and it has no close relatives. But the *pannonica* series of perennials and the *argolica* group of annuals were probably derived from an ancestral stock represented by *patula*, which is apparently a relict in which certain parts, especially the pappus, have become very greatly reduced. *Pannonica*, *lacera*, and *chondrilloides* are closely related and fairly primitive species, while *incana* and *taygetica* are polyploids showing considerable resemblance to them. The *argolica* quartet is a very closely related group in which the marked differentiation in gross morphology must have come about through gene mutation.

On the other side of *patula* are *tenuifolia* and the *gymnopus-pterothecoides* group. Evidence that *tenuifolia* has 4 as the basic haploid number will be presented under chromosome morphology. This species is the

## EUCREPIS—PHYLOGENY AND CHROMOSOME NUMBER

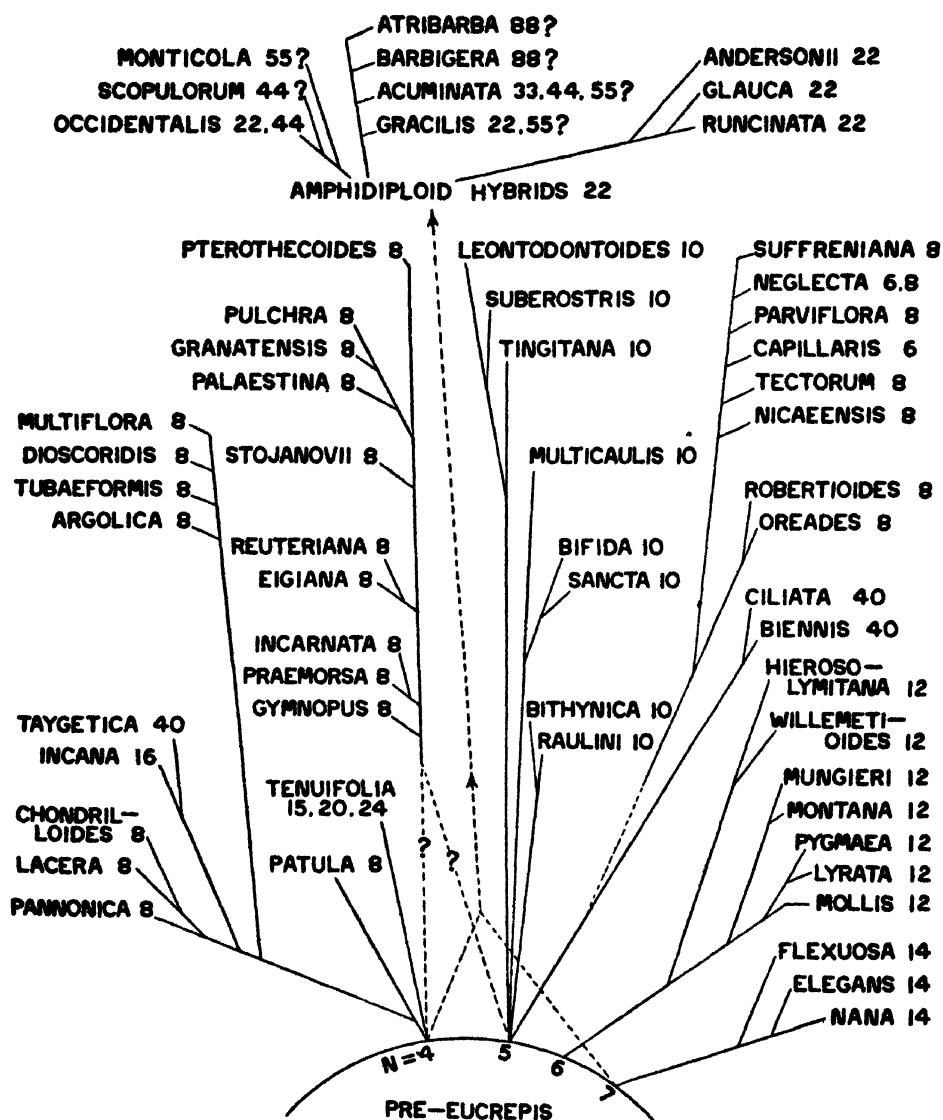


Fig. 4. Phylogenetic relations and chromosome numbers of fifty-nine species in subgenus *Eucrepis*.

only representative, thus far studied cytologically, of an eastern Asiatic group which was probably derived from a 4-paired stock different from the *patula* line. At any rate, that the two lines have diverged widely is

shown not only by gross morphology but also by geographic distribution, since the known representatives of the *patula* line are all restricted to the Mediterranean region.

The *gymnopus-pterothecoides* series is a remarkable group of related species in which reduction in life-cycle and morphological specialization has proceeded without change in chromosome number and without much change in chromosome morphology, as will be shown below. The lower five species in this series are perennials with relatively unspecialized fruits; while the upper five are annuals which are all more specialized, especially in fruit characters, than the perennials. The most extreme example of such specialization is found in *pterothecoides*, in which the achenes are shortly beaked and this plant, unlike all the others, is very precocious and short-lived. These ten species, therefore, provide a beautiful example of an evolutionary series in which, by elimination, it must be concluded that the genetic process making evolution possible is gene mutation. The problem of derivation of this interesting group, however, involves the possibility of transformation in chromosome number from 10 to 8. This will be discussed under chromosome morphology. For the present it is sufficient to indicate by the broken lines and question marks that the group may have come from either a 4-paired or a 5-paired progenial stock.

The *oreades-suffreniana* assemblage, with  $n = 4$  for base number, as treated here, also involves hypothetical derivation from a stock with  $n = 5$  as base number. This hypothesis is supported by two lines of evidence. First, *biennis* with about forty chromosomes has been proved by cytogenetic study (Collins and Mann, 1923) to be an octoploid species and its close relative, *ciliata*, has the same number of chromosomes. Second, *nicaeensis*, with eight chromosomes, is a biennial species and is so similar in general appearance to *biennis* as to make it difficult for anyone but an experienced student to identify herbarium specimens of the two species. The annual species, *tectorum*, also shows considerable resemblance to *nicaeensis* and *biennis*, and the other four annuals, *capillaris*, *parviflora*, *neglecta*, and *suffreniana*, are progressively farther removed. The alpine perennials, *oreades* and *robertioides*, each with eight chromosomes, are thought to be more primitive representatives of the same 4-paired stock that produced the *nicaeensis* group.

The 10-chromosome *Eucrepis* species appear in the central part of the diagram. Only eight such species have been reported thus far, but there are several related species which have not been available for cytologic study. These eight species comprise three diverse groups which probably represent different lines in the immediate ancestry although these lines converge and probably originated in a common progenial stock. The most primitive group contains *raulinii* and *bithynica*, which are alpine perennials with a woody caudex as in the 4-paired

species, *oreades* and *robertioides*; this fact gives added weight to the hypothesis that the latter originated from a 5-paired ancestral stock.

The *multicaulis* and *sancta-bifida* assemblage is extraordinarily interesting in that *sancta* and *bifida* represent a group of about ten species which have hitherto been classified under the genus *Pterotheca* although Hooker (1882) expressed the opinion that this group should be merged with *Crepis*. Since the one character supposed to distinguish *Pterotheca* from *Crepis* (bristle-like paleae on the receptacle) is sometimes absent, Hooker's opinion appears to be sound, and now that evidence both morphological and cytological establishes the relationship of these two species with *Crepis multicaulis* this opinion is confirmed. The question of relative position in the phylogenetic series is somewhat complicated. *Sancta* and *bifida*, because of their annual habit and dimorphic fruits, would be considered more specialized than *multicaulis*; but reduction in size of flowers and fruits has gone much farther in *multicaulis*. The position of these three species as represented in figure 4 is largely a matter of convenience.

*Crepis tingitana*, a native of Morocco and Spain, is in certain respects a primitive species. At any rate it shows strong resemblance to certain African species of *Catonia*. In shape of achenes, however, it is variable, certain forms having the achenes definitely though coarsely beaked. From external morphology alone it would seem unlikely that *tingitana* arose from the same 5-paired stock as the preceding species. At the same time it shows sufficient resemblance to *suberostris* and *leontodontoides* to warrant the inference that they may have arisen in the same ancestral line. But the two latter species are more highly specialized, particularly *suberostris*, which is an annual; while both include forms with *Barkhausia*-like achenes.

The seven 6-paired species comprise a well marked yet much diversified group. *C. mollis* is evidently the most primitive; then come *lyrata* and *pygmaea*; then the two pairs of closely related species, *montana* and *mungieri*, *willemetioides* and *hierosolymitana*. There are no closely related groups, so it appears that they arose from a distinct ancestral stock. But certain peculiarities in the morphology of their chromosomes remain to be considered.

The three 7-paired *Eucrepis* species, *nana*, *elegans*, and *flexuosa*, are also closely related to one another and seem to have arisen from a distinct ancestral stock. There are good reasons for thinking that these low-growing perennials are some of the remaining representatives of a comparatively ancient group. In fact, the most diminutive one, *C. nana*, is also the most widely distributed species in the entire genus, extending from central Asia across the northern hemisphere to northeastern North America. Such evidence as this lends support to the hypothesis which has been advanced (Hollingshead and Babcock, 1930) that 7-paired

species must have hybridized with certain 4-paired species and produced through amphidiploidy the 22-paired American species and their polyploid relatives shown at the top of figure 4 (cf. fig. 15b).

### BARKHAUSIA

The phylogenetic relations of thirty-two species of *Barkhausia* and their chromosome numbers are shown in figure 5. In general the most primi-

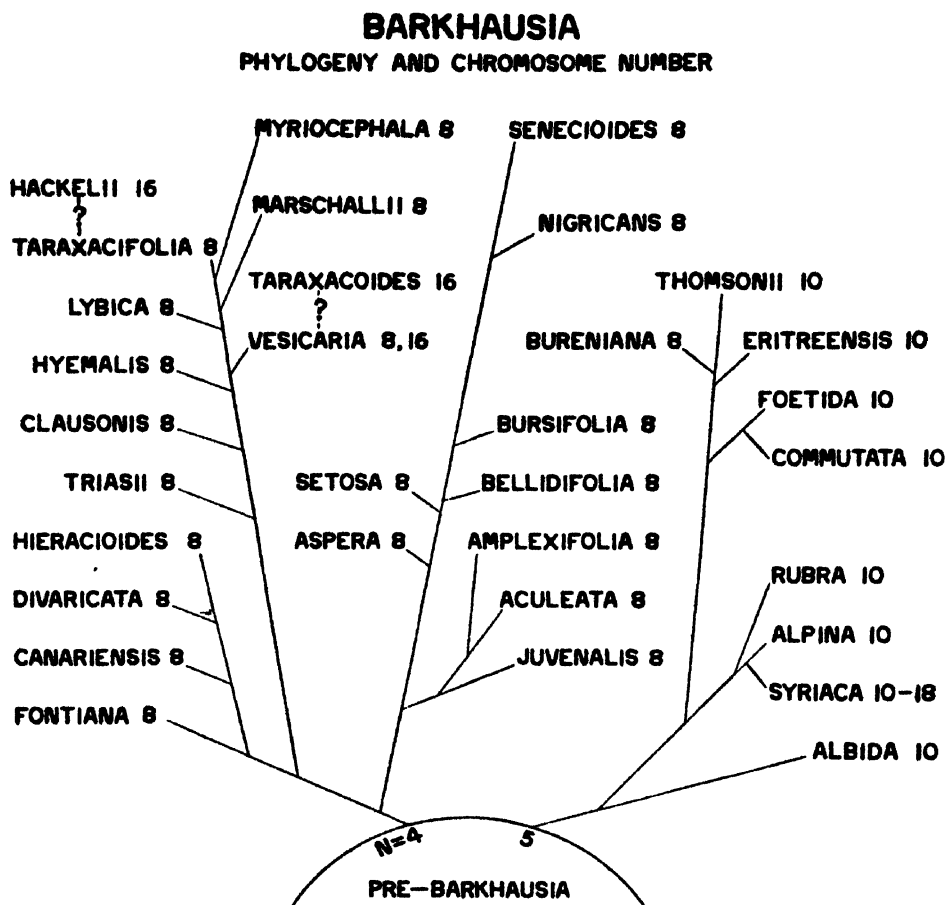


Fig. 5. Phylogenetic relations and chromosome numbers of thirty-two species in subgenus *Barkhausia*.

tive species are near the base and the most specialized at the top. The species having ten chromosomes, shown on the right, include one perennial, *C. albida*, of southwestern Europe and Morocco, which consists of six subspecies and which is one of the most primitive specific groups in the entire genus. There is good morphological evidence of its fairly close relationship to *C. alpina* of Asia Minor, and the associated species, *C. syriaca*; also, though less closely, to *C. rubra* of southern Europe;

while farther removed but still clearly connected is the *commutata-thomsonii* group of southern Europe, Asia Minor, northeast Africa, Persia, and India. Allied with the latter, especially with *C. thomsonii*, is the 8-chromosome species, *C. bureniana*. The morphological evidence of this relationship is indisputable. The question of chromosome morphology is discussed below.

The 8-chromosome *Barkhausia* species include three distinct series which seem to have a common origin. Below on the left are four perennial species, *fontiana* of western Morocco, *canariensis* of the Canary Islands, and *divaricata* and *hieracioides* of Madeira. They exhibit considerable morphological similarity. *Fontiana* and *canariensis* especially must be recognized as fairly primitive types of *Barkhausia*. Next to these is the *triasii-myriocephala* assemblage of the Mediterranean Islands and bordering countries. Of these, *triasii*, *clausonis*, *hyemalis*, and some forms of *vesicaria* are perennials, while the others are annuals which sometimes behave as perennials under favorable conditions. *C. myriocephala* is unquestionably the most specialized member of this series through reduction in size of flower heads, florets, and fruits, although the plant and its basal leaves are very large. The assumed derivation of *hackelii* from *taraxacifolia* and of *taraxacoides* from *vesicaria* is considered in connection with chromosome morphology.

The remaining series of 8-chromosome *Barkhausia* species, all of the Mediterranean littoral, are annuals except *bellidifolia* and *bursifolia*, which, although rather specialized through reduction in size of plant, flowers, and fruits, have retained the perennial habit. *C. juvenalis*, *aculeata*, and *amplexifolia* are obviously closely related, being characterized by having two distinct types of achenes which are similar in all three species. *C. aspera* and *C. setosa* also have dimorphic achenes, but they differ from each other and from the *juvenalis* group in important characters of the fruits. The two remaining species, *nigricans* and *senecioides*, are most highly specialized through reduction throughout the whole plant. They are very precocious, short-lived annuals.

## CHROMOSOME MORPHOLOGY

The general aspects of chromosome morphology in *Crepis* have been discussed in earlier publications (cf. Hollingshead and Babcock, 1930; Babcock and Navashin, 1930). The existence of comparable pairs of chromosomes in different *Crepis* species was first noted by Navashin (1925) and designated as follows: A, long chromosome with longest proximal arm; B, long chromosome with next longest proximal arm; C, usually a shorter chromosome with shorter proximal arm, but sometimes there is little difference between B and C; D, satellite-bearing chromosome; E, a shorter chromosome with median constriction. In the

following illustrations the same order of arrangement of the members of the haploid genom has been followed throughout. This order, from left to right, is A, B, C, D, E, in 5-paired species. Diploid species with more than five pairs often have two or more pairs of E chromosomes, but sometimes B or C seems to be duplicated. The 4-paired species lack E; the 3-paired species, *capillaris*, lacks B; the other 3-paired species, *fuliginosa*, lacks C.

#### CATONIA

Cytological studies have been completed on fifteen of the seventeen species represented in figure 3. The haploid genomes of the three 5-paired species and of two of the 6-paired species are shown in figure 6. In the



Fig. 6. Species of *Catonia* with  $n = 5$  and 6.

former a fairly close resemblance between *sibirica* and *pontana* will be seen in all five chromosomes, the chief difference being in the length of the proximal arms of A and B. This close correspondence is in agreement with the morphological evidence that these species are among the most primitive of the genus, although distinct in many characters and occupying widely separated geographic areas. The genom of *aurea* differs strikingly, all the chromosomes being smaller and the C having a very short distal arm. *Aurea* is a more recent species, since it exhibits specialization through reduction in size and in the strongly attenuate achenes and the development of much red color in the flowers.

The two 6-paired species are of special interest for several reasons. They are closely similar, yet unquestionably distinct in numerous characters. *Paludosa* is the most widely distributed species of *Catonia*, while *viscidula* is restricted to the northern Balkan states. Furthermore, *paludosa* is more reminiscent of *Hieracium* in habit, habitat, achene shape, and the yellowish brittle pappus than any other species which has been studied cytologically. Yet the number, 12, has not yet been reported in *Hieracium*. These two species therefore appear as one of several small groups which may justifiably be included within *Crepis*, but which verge more or less definitely toward some other genus. Since 12 is not known to occur in *Hieracium* one may fairly question whether it must be looked upon as a primitive number in *Crepis*. Possibly these two 6-paired species were derived from some 5-paired stock. Further study is needed on these two species and on *kashmirica* in order to solve this problem.

The eight 4-paired species of *Catonia* fall naturally into two groups according to chromosome morphology, as is shown in figure 7, and by comparing this with figure 3 it will be seen that this grouping agrees with the arrangement according to morphology, geographic distribution, and the occurrence of natural hybrids. The close correspondence between the chromosomes of *blattarioides* and *alpestris* is the more remarkable in view of the marked morphological differences between these two montane species of southern Europe. *C. alpestris* also occurs in Asia Minor, which adds weight to the assumption that it had a common origin with *C. hypochaeridea* of South Africa. The *hypochaeridea* genom has a strong resemblance to that of *alpestris* and there is a general morphological resemblance between the two plants. The Moroccan *C. hookeriana*, a plant of the Grand Atlas Mountains, also resembles *C. alpestris*, though less closely than *hypochaeridea*, and its chromosomes differ more, especially B and C. From the size of the chromosomes it would appear that these four species are of approximately equal age. The slightly smaller size in *hypochaeridea* is in agreement with the evidence from geographic distribution that it is somewhat more recent than the other three.

Strong similarity in size and shape also appears in the genoms of *conyzaefolia*, a species distributed from southern Europe to central Asia, and of *burejensis* and *chrysantha* of eastern Asia. The three are similar morphologically, as are their genoms, but *burejensis* is slightly more specialized than *conyzaefolia*, and *chrysantha* is much more reduced throughout. *C. polytricha* has been confused with *C. chrysantha*, from which it is easily distinguished by the larger, ventricose involucre and yellow indumentum and by other characters. Critical study of the chromosomes of *polytricha* has been difficult because of limited material. From available evidence it appears almost certain that this species is



an autotetraploid, but the marked differences between *polytricha* and *chrysantha* in the A and D chromosomes indicate that if *polytricha* did originate from *chrysantha* through polyploidy, the event was not of recent occurrence. This is consistent with the morphological differences between the plants and the wide geographical distribution of *polytricha*.



Fig. 7. Species of *Catonia* with  $n = 4$  and 8.

Genoms of *C. bungei* and *C. crocea* of *Catonia* are shown in figure 8 in comparison with that of *C. oreades* of *Eucrepis*. Morphologically, *C. crocea* is either intermediate between the two diploid species or exceeds them both in certain quantitative characters. The geographic distribution of the three species is in excellent agreement with the hypothesis that *crocea* originated as an amphidiploid hybrid between the other two. Genetic evidence is limited to data on some  $F_1$  hybrids between *bungei*

and *crocea*. These hybrids were intermediate between the two species and exhibited a low degree of fertility. Chromosome morphology agrees fairly well with the foregoing hypothesis, although the chromosomes of *oreades* and *bungei* are too similar to make the evidence definite. In arranging the haploid genom of *crocea*, in each of the four sets of two chromosomes the one believed to correspond with *bungei* is shown on the

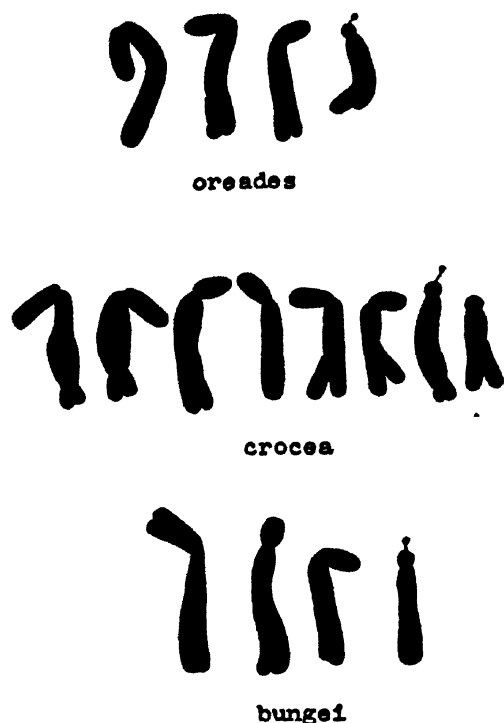


Fig. 8. Cytological evidence on the origin of *Crepis crocea*.

left. The absence of a satellite from the "*oreades*" D chromosome in *crocea* seems to be constant—another example of amphiplasty (Nava-shin, 1928).

#### EUCREPIS

The genoms of the 10-chromosome *Eucrepis* species are presented in figure 9. It will be recalled that *raulinii* and *bithynica* are rather primitive types which may have arisen from the same 5-paired ancestral line as the other six species. The close correspondence in shape among all eight genoms agrees with this conception. The full significance of this evidence can hardly be appreciated without a somewhat detailed comparison of the morphology of the plants (pp. 297 f.). The high degree of reduction and specialization which has taken place in the *multicaulis* group seems to have been accompanied by notable reduction in size of the chromosomes. *Tingitana* also is a rather primitive species, while

*leontodontoides* is much more specialized in several characters. Here again there is notable difference in size of the chromosomes. *Suberostris*, however, seems an exception to the general rule since its chromosomes



Fig. 9. Species of *Eucrepis* with  $n = 5$ .

are large, although it is a considerably reduced annual. In fact it fits less well in this series than the other species, not only with reference to its chromosomes, but also in its external morphology; but it has no closer relatives.

*Crepis patula* and its nearest relatives are represented by the haploid groups of chromosomes shown in figure 10. That the relationship is not

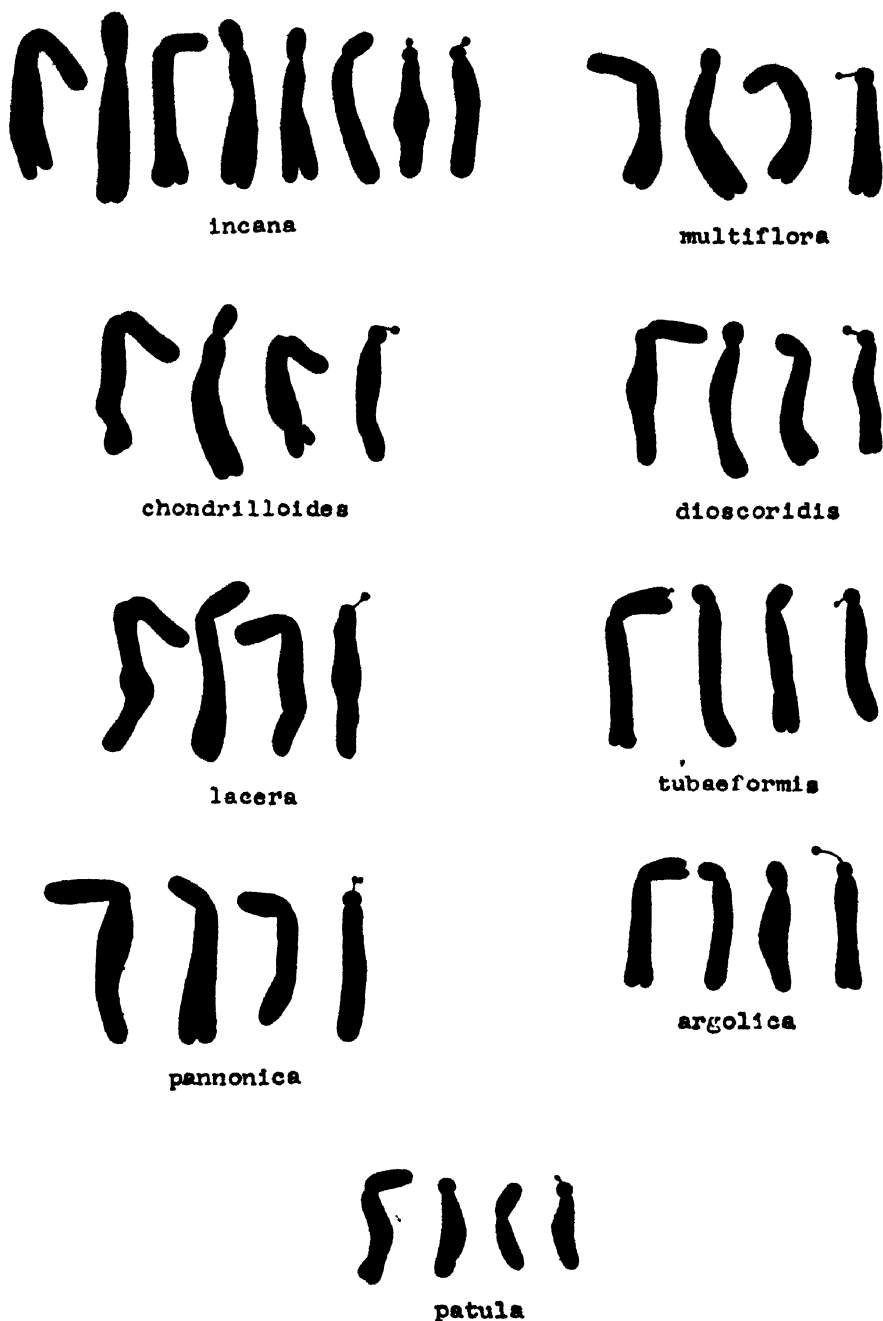


Fig. 10. Species of *Eucrepis* with  $n = 4$ .

close is indicated by the fact that the *patula* genom does not correspond entirely with the chromosome types in either of the two series. It is noteworthy, however, that the B chromosome in *patula* corresponds with B

in the *argolica* series, while *patula* C is more like C in the *pannonica* series. This may indicate a common ancestry and is in keeping with the evidence that *patula* is really an old species which is still primitive in the greater number of characters but has become greatly reduced in a certain part. It is not unlikely that, in its comparatively long existence as a species, there has also been some reduction in the size of its chromosomes.

The close similarity of the *pannonica*, *lacera*, and *chondrilloides* genomes is to be expected from their common morphological features. It may be noted that *chondrilloides* is the most restricted in distribution and the most specialized of the three, and that its chromosomes appear to be slightly though perhaps not significantly smaller. That *incana* is an autotetraploid appears fairly certain from its B, C, and D chromosomes, and the apparent unlikeness in the A's may not be an actual difference. The diploid species from which it may have originated has not yet been discovered. *C. taygetica* may be mentioned here as another polyploid species, of which the diploid ancestor is unknown. Evidence from morphology and geographic distribution places it in this series. Incomplete study of its chromosomes indicates that it is a high polyploid of some sort, possibly a decaploid. The presence in somatic tissue of forty chromosomes which correspond in size to those of other species in this group lends considerable weight to this assumption. Only three chromosomes were observed which bore unmistakable satellites, but several others were present which might be D chromosomes and it is frequently found that, in a large genome such as this, all the D's do not possess a satellite. The *argolica* series of very closely related species from Greece also exhibit great uniformity in chromosome types. The only point of importance is the inconsistency with the general rule that more highly specialized and reduced species have smaller chromosomes, since *multiflora* is such a species, while *argolica* is certainly the most primitive of the four.

*Crepis tenuifolia* was reported by Hollingshead and Babcock (1930) as having fifteen chromosomes. This report was based on eight plants, grown from wild seed, of one accession from Mongolia. Since then we have counted the chromosomes of thirty-three plants, grown from wild seed, of a different accession also from Mongolia. Of these, thirty-one had fifteen chromosomes and two had twenty-four chromosomes as the diploid number. It appears that most Mongolian plants of this species have fifteen chromosomes, the odd number being maintained through some form of apomictic reproduction, but that sexual reproduction occasionally takes place, producing plants with higher numbers. More recently an accession of this species has been received from Kashmir, which has  $2n = 20$ . In figure 11a is shown a diploid group with fifteen chromosomes, and b is the haploid genome in which each of the eight

types represents a pair except the one at the right end, which is the odd member. It will be noted that there are two pairs of A's, B's, and D's in the diploid complex. Figure 11c, d presents the haploid genom of the Himalayan form of the species, in which each type shown in c represents a pair and the four chromosomes in d are odd. Referring to the haploid genom, there are certainly two types of D chromosomes and

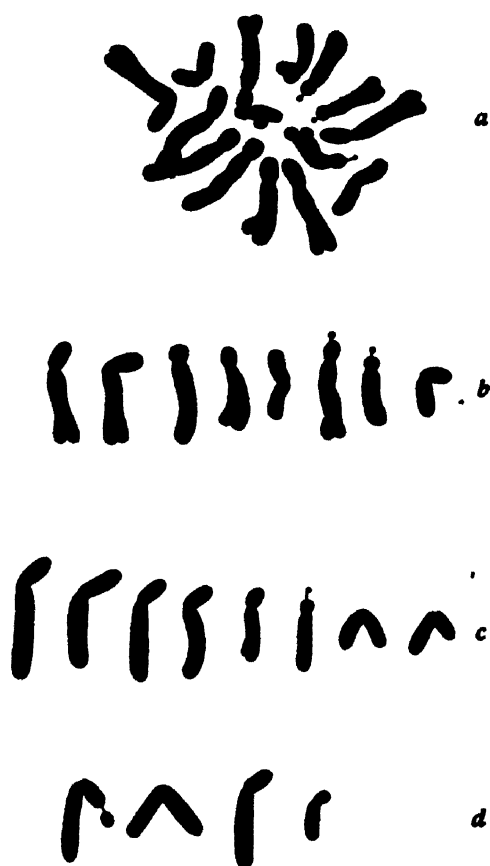


Fig. 11. *Crepis tenuifolia*: a, diploid genom of the 15-chromosome form; b, one member of each of the seven pairs in diploid genom and, at the right end, the odd chromosome; c, one member of each of the eight pairs in a 20-chromosome form; d, the four odd members in the same genom.

presumably also of A's, B's, and C's or E's. Hence it appears certain that the species originated as an amphidiploid hybrid and that the odd number, 15, is maintained by some form of apomictic reproduction, although sexual reproduction sometimes occurs producing such forms as the 24-chromosome plants already mentioned. The 20-chromosome form may also be a segregation product resulting from sexual reproduction. Further studies are in progress on this remarkable species.

In figure 12 are depicted the highly uniform genomes of the ten species in the *gymnopus-pterothecoides* series. The rather wide morphological

*reuteriana**pterothecoides**eigiana**pulchra**incarnata**granatensis**praemorsa**palaestina**gymnopus**stojanovii*

Fig. 12. Species of *Eucrepis* with  $n = 4$ .

differences among the members of this group must depend upon genic diversity. The extent of some of these differences is indicated from the fact that four of the species were originally classified and described under

three other genera, *Hieracium*, *Cymboseria*, and *Phaegasium*. Another was named for *Pterotheca* although the resemblance is only superficial. An interesting difference in chromosome morphology is the distal satellite on the C chromosome in *pulchra*, in its close relative, *granatensis*, and in *pterothecoides*, which resembles *pulchra* more than it does any other member of the series, although it is a distinct species. Since the preparation of this figure similar distal satellites have been discovered in *palaestina* and *reuteriana*, which are more closely related to *pulchra* than are the remaining species. The possibility that the genome characterizing this group was derived from some 5-paired ancestor has been mentioned. The suggestion comes from the fact that equiarmed A chromosomes are unique in *Crepis*, being found in only two other series (cf. figs. 14 and 15). If the putative ancestor had J-shaped A's and a pair of E's, it is conceivable that reciprocal translocation between A and E, followed by meiotic irregularities, might result in the V-shaped A chromosomes and elimination of the rest of the E chromosome, thus establishing this 4-paired type. Translocations between nonhomologous chromosomes, such as might lead to the origin of new chromosome numbers, have been observed in animals and plants, and Navashin (1932) has proposed a hypothesis of evolution of chromosome numbers based partly on the observation of such phenomena in *Crepis*.

The *oreades-suffreniana* series is represented in figure 13. There is fairly close correspondence between the genomes of *oreades* and *tectorum* and the two species occur in the same region of northern Asia. Although *robertioides* and *parviflora* are less similar in their haploid genomes, they both occur in Asia Minor and are probably distantly related. Both *oreades* and *robertioides* are woody-based perennials and in other respects also are more primitive than the other species in this series. Furthermore, *oreades* is much more primitive than *robertioides*. The other species are annuals (*nicaeensis* is often biennial) and progressive reduction in size of plant and parts reaches a climax in the low, delicate, short-lived forms of the *neglecta-suffreniana* group. Corresponding reduction in size of the chromosomes is very notable in this series. *Crepis capillaris* must now share its distinction as a 3-paired species with *fuliginosa*. In the latter it seems to be the C chromosome which is lacking, while in *capillaris* it is the B, but the distinction between B and C chromosomes is an arbitrary one. It may be significant, however, that the A and D chromosomes are present in both of these plants.

*Crepis neglecta*, *sensu lato*, presents a unique situation in respect to the chromosomes. In taxonomic treatments of this assemblage both *cretica* and *fuliginosa* have been classified in subspecific categories under *neglecta*. The morphological resemblances existing among the three entities are perhaps sufficient grounds for such a systematic treatment. In adopting it, however, it must be recognized that the divergence in chro-



mosome number, size, and shape is unusually great for a single species. With this frank admission there would seem to be no serious objection to such classification. For the cytological criterion is not of paramount

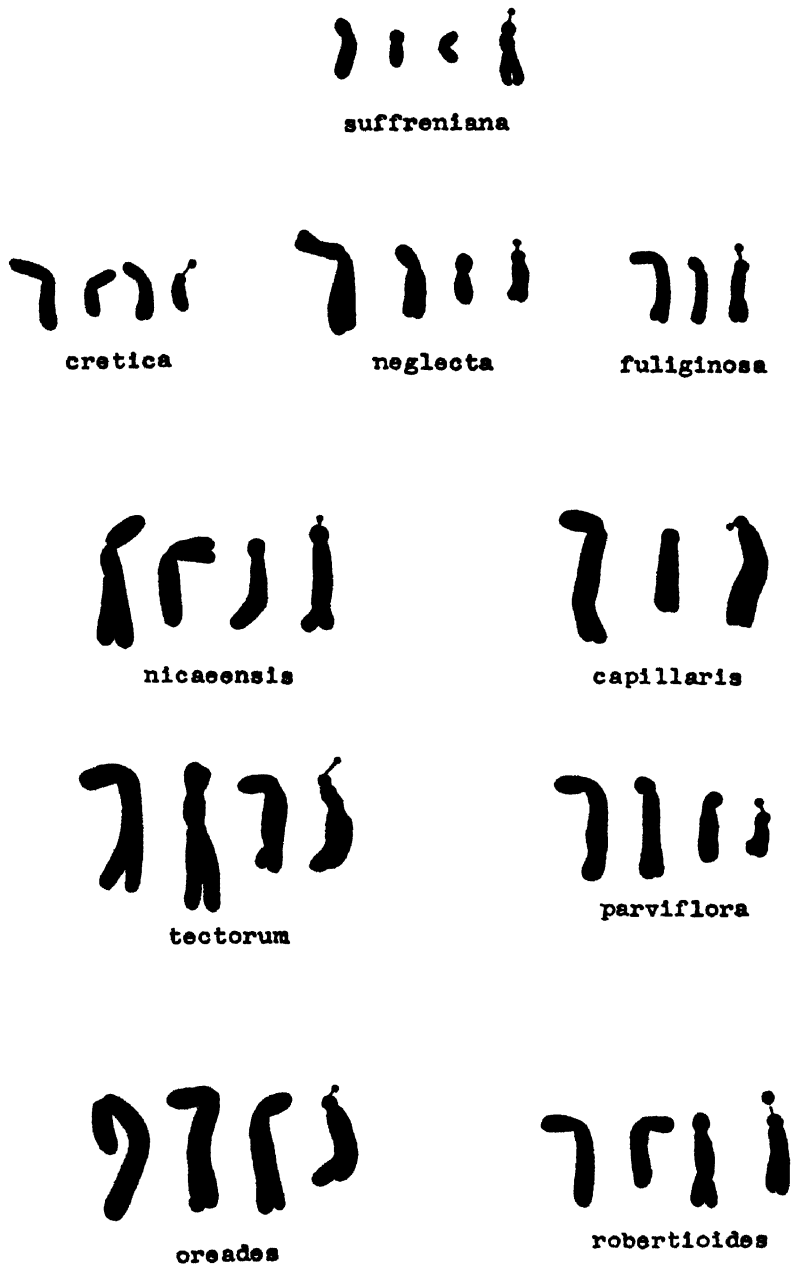


Fig. 13. Species of *Eucrepis* with  $n = 4$  and 3.

importance: in spite of the differences in number and morphology of the chromosomes, essentially the same residual complement of genes may be present in all three entities. Critical comparison, however, reveals a num-

ber of significant morphological differences and these, together with the outstanding chromosomal differences, will justify recognition of the three as distinct, though very close, species. *C. suffreniana*, while closely related to *neglecta*, is beyond doubt a distinct species and its A and B chromosomes are notably different from those of *neglecta*, *fuliginosa*, and *cretica*.



Fig. 14. Species of *Eucrepis* with  $n = 6$ .

Preliminary study of the haploid genomes of *C. biennis* and *C. ciliata*, in both of which  $2n = \pm 40$ , indicates that they are certainly octoploids, based on  $n = 5$ , as has been previously reported for *C. biennis* (Collins and Mann, 1923). In both species there are two sizes of D chromosomes which may indicate hybrid origin. Further study is reported elsewhere (Babcock and Swezy, 1934).

Figure 14 shows the haploid genomes of the 6-paired *Eucrepis* species. The species are arranged in the same relative positions as shown in figure 4, these positions being determined on the basis of comparative

morphology, as summarized earlier, and geographic distribution. *C. mollis* extends from western Europe to middle Russia; *pygmaea* occurs only in the European Alps, *montana* only in Greece, and *mungieri* only in Crete; while *lyrata* is found in western Siberia, *willemetioides* in northeastern Persia, and *hierosolymitana* in Palestine, Syria, and Cyprus. The wide distribution of the series as a whole and of its most primitive member indicates relative antiquity, and necessitates the acceptance of 12 as a primitive number in *Eucrepis*, unless it be assumed that these species originated through hybridization between 5-paired species, followed by amphidiploidy and consequent transformation and elimination of certain chromosomes. This assumption is well supported by the following comparative classification of chromosome types in the haploid genomes of the seven species.

*mollis* 2 A (1 V, 1 compound), B, C, 2 D lacking satellite.

*pygmaea* 2 A (both V's, 1 with satellite), B, 2 C, E.

*lyrata* 3 A (1 V, 2 with satellite), 2 C, E.

*montana* 2 A (1 V), B with satellite, 2 C, E.

*mungieri* A, B, C†, D, 2 E.

*willemetioides* 2 A (1 V, 1 with satellite), B, C, 2 E.

*hierosolymitana* 2 A (1 V, 1 compound), B, C, D lacking satellite, E.

The foregoing classification is made by comparing each chromosome with the characteristic types in a basic 5-paired genom; it does not depend on the order of arrangement within the haploid groups shown in figure 14. The presence of E chromosomes in all but one of the seven species, the duplication of E, D, C, and A chromosomes, and the striking alterations of A chromosomes in most of these species, all strongly indicate hybrid origin and that the parental species involved had five pairs of chromosomes.

The genomes of the 7-paired *Eucrepis* species are shown in figure 15. The general similarity of the chromosome types is in agreement with the evidence from external morphology indicating that these are closely related species. But there are notable differences among the first three chromosomes from the right end of each haploid group. Like the 6-paired species this is a widely distributed and relatively ancient group, the members of which have become much reduced and considerably specialized concomitantly with their adaptation to the rigors of alpine and arctic environments. This seems to indicate that 14 is also a primitive number in *Crepis*. But here also it is not difficult to imagine that these species originated through hybridization of 5-paired species plus amphidiploidy, followed by transformation and elimination of some chromosomes. The presence of E chromosomes and more than one pair of certain chromosome types in all three species strongly supports this hypothesis. Two species are known from very high altitudes in the Himalaya Mountains. These might have been the parents of this group, but

unfortunately they have not been studied cytologically. Even if they should not have the proper chromosome number and morphology, however, this would not greatly discount the hypothesis here advanced. The possibility should also be noted that both putative parents were 4-paired species and that these 7-paired species are modified derivatives from a 16-chromosome amphidiploid. At any rate it is hardly justifiable to con-

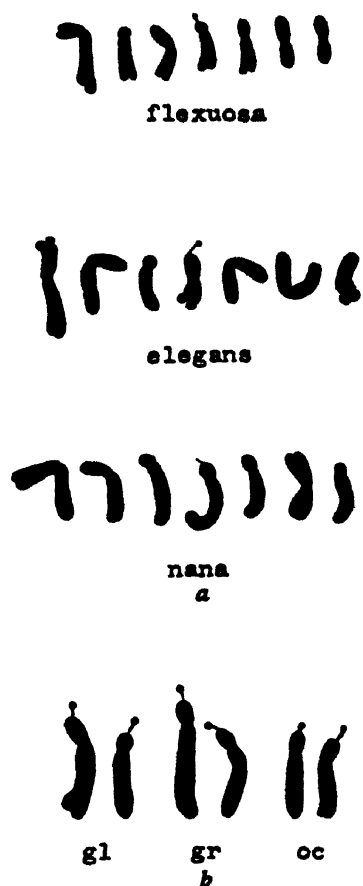


Fig. 15. *a*, Species of *Eucrepis*, with  $n = 7$ ; *b*, representatives of the two pairs of D chromosomes of *glauca* (*gl*), *gracilis* (*gr*), and *occidentalis* (*oc*), indicating origin of all three groups of American species with  $n = 11$  through interspecific hybridization and amphidiploidy.

clude that either 12 or 14 is a truly primitive number in *Eucrepis* until it is shown that the species under discussion could not have originated through interspecific hybridization and amphidiploidy.

Analysis of the distribution of chromosome types in the octoploid and decaploid species has not been attempted. In some of the 22-chromosome American species, however, there is definite cytological evidence indicating the manner of their origin. It is noteworthy that the number, 22, could not have arisen by autotetraploidy from any haploid number known in *Crepis*, although it is conceivable that autotetraploids with

twenty or twenty-four chromosomes might have produced 22-chromosome derivatives. But preliminary studies of the genomes of some 22-chromosome species indicate that they cannot be autotetraploids. This is most clearly demonstrated by comparison of the two pairs of satellite-bearing chromosomes found in each of these species. In figure 15*b* are shown representatives of the two pairs of D chromosomes from each of three species, *glauca*, *gracilis*, and *occidentalis*, representing the three subgroups of this assemblage. In *occidentalis* the difference is slight but in *glauca* and *gracilis* there are obvious differences in size of the two pairs. This evidence, together with 11 as the haploid number for ten species, is sufficient proof that all the American species except *nana* and *elegans* originated through hybridization of 8- and 14-chromosome species followed by amphidiploidy.

#### BARKHAUSIA

The 10-chromosome *Barkhausia* species are compared with reference to their haploid genomes in figure 16. It will be recalled that *albida* is certainly the most primitive member of this series and that *alpina* is its nearest relative. The proximal arm is longer in the A and C chromosomes of *albida*; also the D and E chromosomes are larger in this species. *C. syriaca* is believed to have originated through hybridization of two *alpina* subspecies followed by genic mutation and chromosomal modification. Indigenous plants possess supernumerary chromosomes, mostly of one type, which is not shown here as part of the basic haploid genome for reasons advanced by Cameron (1934). The basic genome of *syriaca* resembles closely the haploid group of *alpina*. These two closely related species are natives of Asia Minor and the Caucasus while *rubra* occurs in Crete, the southern Balkans, and Italy. *C. rubra* must be considered a more recent species because of reduction in size of plant and especially because of its pink flowers as contrasted with the yellow flowers which occur in all other *Barkhausia* species. Furthermore, its scape-like flower stems point to some species other than *alpina* or *albida*, although this species doubtless arose from the same ancestral stock as the two latter. It is not surprising, therefore, to find some striking differences in chromosome morphology in *rubra*, but the larger size of its chromosomes makes it another illustration showing that reduction in size of the chromosomes does not always accompany higher development. The *commutata-thomsonii* series has a markedly uniform type of genome, as would be expected from the similar morphology of the four species. The genome of *C. bureniana* is included in figure 16 because, on morphological grounds, this species is certainly related to *foetida* or *thomsonii* and because, judging from geographical distribution, the latter is probably its closest relative. Its chromosomes, however, resemble those of *alpina* more than those of *thomsonii* although the genomes of the

two latter species are undoubtedly similar. This suggests several possible modes of origin for *C. bureniana*, but these are all too vague to justify further discussion here. An investigation of this species is in progress.

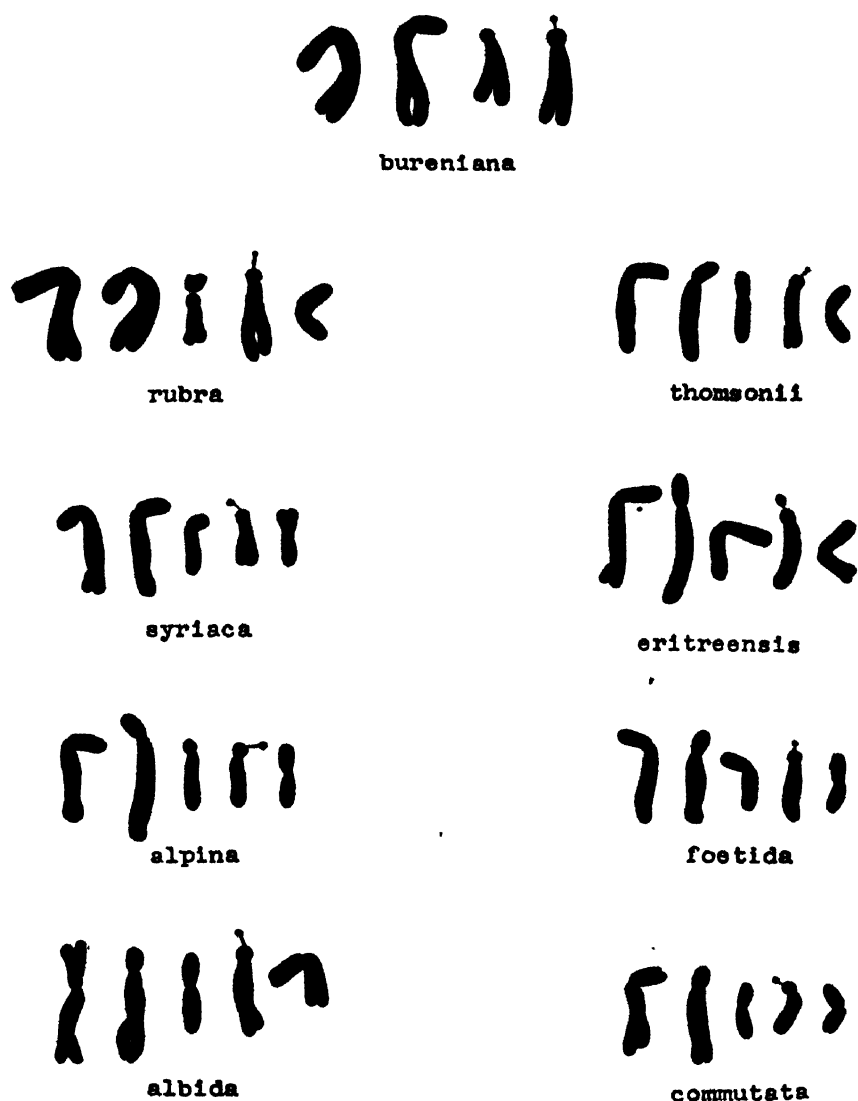


Fig. 16. Species of *Barkhausia* with  $n = 5$  and 4.

In figure 17 are shown the genoms of two related *Barkhausia* series. The strong general resemblance of the genoms is the most striking thing in this illustration and from the external morphology of these species it seems certain that they all arose from the same ancestral stock. The lower four are the more western species, of which *fontiana* and *canariensis* are recognized as more primitive; and the chromosomes of *fontiana* are definitely larger than any of the others. Of the remaining nine spe-

hackelii taraxacoides

hackelii

taraxacoides

taraxacifolia myriocephala vesicaria

taraxacifolia

myriocephala

vesicaria

lybica nyemalis

lybica

nyemalis

triasii clausonii

triasii

clausonii

canariensis hieracioides

canariensis

hieracioides

fontiana divaricata

fontiana

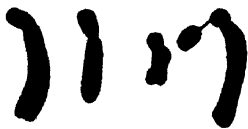
divaricata

Fig. 17. Species of *Barkhausia* with  $n = 4$  and 8.

cies, the lower four are more primitive and their chromosomes are somewhat larger. *C. vesicaria*, *myriocephala*, *marschallii*, and *taraxacifolia* are very closely related and their chromosomes show the closest similarity. The *marschallii* genom is not illustrated. This species is most closely related to *taraxacifolia* and its chromosomes resemble those of that species closely, except the B, which is more like the B of *vesicaria*. That *C. taraxacoides* is an autotetraploid is clearly indicated by the virtual identity of its corresponding pairs of A, B, C, and D chromosomes; and they resemble those of *vesicaria* so nearly as to suggest this as the parent species. Furthermore, autotetraploid forms of *vesicaria* have been discovered (table 1) which resemble the typical diploid form rather closely except in size throughout. But *taraxacoides* differs from *vesicaria* in certain important characters, particularly in the involucre. Therefore, if *taraxacoides* did spring from *vesicaria* through autotetraploidy, it was not a recent event. In *hackelii* the A, B, and D chromosomes are sufficiently unlike to suggest origin through amphidiploidy with *taraxacifolia* as one parent. But no other species is known which, by its morphology and native habitat, could have been the other parent. Therefore, it seems more probable that *hackelii* also originated through autotetraploidy and that there has been some chromosomal alteration.

The remaining 4-paired *Barkhausia* species are represented in figure 18. There can be no doubt that *aculeata*, *juvenalis*, and *amplexifolia* are closely related species and that the last is the most highly specialized. Its chromosomes are much smaller than those of the other two. The C chromosome of *juvenalis*, however, is smaller than that of *aculeata*, yet *juvenalis* is less reduced in size of heads and achenes than *aculeata*, though in size of plant it is smaller. In comparing the chromosomes of *aspera* and *setosa* it may be noted that the "A" and "B" of *aspera* might well be interchanged; they would then compare fairly closely with the A and B of *setosa*. At any rate, the most striking differences in the two genoms are found in the C and D chromosomes. In size of heads and achenes *setosa* is more reduced than *aspera*, yet it has about the same total chromosome length. The genoms of the two perennial species are closely similar and they are probably about equal in age, although the fruits of *bursifolia* have become much more reduced and specialized, with a relatively long delicate beak, than those of *bellidifolia*. The same is true of *senecioides* and *nigricans* except that, in the former, reduction in size of fruits and specialization of the beak is even more extreme, and its chromosomes are definitely smaller. The notable difference in each of the four chromosomes is consistent with the morphological evidence that the two species are not closely related. In fact, from the achenes alone the closest relative of *senecioides* is *bursifolia* and the chromosomes of the two species are similar except in size.



*senecioides**nigricans**setosa**bursifolia**aspera**bellidifolia**aculeata**juvenalis**amplexifolia*Fig. 18. Species of *Barkhausia* with  $n = 4$ .

## SUMMARY AND CONCLUSIONS

1. *The genus*.—*Crepis* is a natural group of more than two hundred species, distributed widely in the northern hemisphere and Africa. Some of them are common and well-known plants while many are extremely rare, little known, or occur only in relatively inaccessible places. In the past decade one hundred seven species of *Crepis* have been obtained in living condition from all parts of the world and examined cytologically. The present paper combines these cytological data with other evidence bearing on phylogenetic relationship.

2. *The subgenera*.—*Catonia* with about one-fourth, *Eucrepis* with about one-half, and *Barkhausia* with the other fourth of the species, are the major natural subdivisions of the genus. They are characterized in the order just given by progressively greater specialization of the involucre and fruits, and along with this differentiation goes generally reduction in length of life-cycle and in size of the plant and its parts.

3. *Chromosome numbers in Crepis*.—The series of characteristic diploid numbers found in the Old World species is 6, 8, 10, 12, 14, 16, 40, and in North American species 22, 33, 44, 55(?), 88(?), besides 14 in two representatives of an Old World group. Occasional irregularities in these characteristic numbers occur and several species are known to have variable numbers.

4. *Chromosome numbers in the subgenera*.—The number of species having a given chromosome number being indicated by an exponent, the distribution of chromosome numbers of Old World species is as follows: *Catonia*, 8<sup>8</sup>, 10<sup>8</sup>, 12<sup>8</sup>, 16<sup>8</sup>; *Eucrepis*, 6<sup>2</sup>, 8<sup>25</sup>, 10<sup>6</sup>, 12<sup>7</sup>, 14<sup>8</sup>, (15–24)<sup>1</sup>, 16<sup>1</sup>, 40<sup>3</sup>; *Barkhausia*, 8<sup>22</sup>, 10<sup>8</sup>, (10–18)<sup>1</sup>, 16<sup>2</sup>. The American species are all of *Eucrepis*.

5. *The primitive numbers*.—Although 8 is the most prevalent diploid number in the genus, 10 must be considered more primitive than 8 because (1) the most primitive species in the genus, such as *sibirica*, *pontana*, and *albida* have 10; (2) no species with ten chromosomes are as greatly reduced or specialized as some of the species with eight chromosomes. If the 8-chromosome lines were derived from 10-chromosome ancestors, the most likely process would be by reciprocal translocations between nonhomologous chromosomes, followed by meiotic irregularities leading to complete elimination of one pair of chromosomes. Such a process seems the most probable mode of origin of the 8-chromosome species, *C. bureniana*, and of the two 6-chromosome species, *capillaris* and *fuliginosa*.

6. *Ancient but doubtfully primitive numbers*.—In addition to 10 and 8, the numbers 12, 14, and 16 must be considered as possibly primitive in this genus. In *Catonia* there are three species with twelve chromosomes

which may be diploids, aneuploids, or polyploids; and there are three species with sixteen chromosomes, two of which are polyploids and one is still doubtful. In *Eucrepis* there are seven species with twelve, and three species with fourteen chromosomes. They are widely distributed and fairly primitive, yet it is possible that they are all polyploids of some sort. The one species with sixteen chromosomes is a tetraploid. In *Barkhausia* there are no species with twelve or fourteen and the two with sixteen chromosomes are tetraploids. In view of the small proportion of species having these numbers and the uncertainty that these species are simple diploids, the numbers 12, 14, and 16 cannot be accepted as primitive. At least they are not basic like 8 and 10.

7. *Secondary numbers and modes of derivation.*—Secondary numbers are 6, 12, 14, 15, 16, 22, other multiples of 11, and 40. Each of the two species with six chromosomes, *capillaris* and *fuliginosa*, was probably derived from a 4-paired ancestor. The most probable mode of origin of these 3-paired species has been described (cf. *primitive numbers*). It is conceivable that all the species with twelve and fourteen chromosomes were derived from amphidiploid hybrids. The constantly increasing evidence on the importance of amphidiploidy in the evolution of higher plants and the demonstration that it has played a definite rôle in *Crepis* lend support to this idea. All but one of the 16-chromosome species have been shown to be either autotetraploids or amphidiploids. The 22-chromosome species are the products of interspecific hybridization and amphidiploidy, and the higher-numbered American species are polyploids derived from them. One of the 40-chromosome species, *biennis*, is an octoploid ( $n=5$ ) and the closely related *ciliata* may be one also. The other 40-chromosome species, *taygetica*, is probably a decaploid ( $n=4$ ). Thus there are certainly two general processes by which new chromosome numbers have originated in *Crepis*, namely, by interspecific hybridization with amphidiploidy, and by polyploidy. It is also necessary to assume that some process, such as reciprocal translocation, has led to reduction in number from 10 to 8 and from 8 to 6. The change from 10 to 8 is of basic importance in the evolution of the genus.

8. *Chromosome number and phylogeny.*—Throughout the genus there is close correspondence between chromosome numbers and external morphology of the plants. The most primitive species have ten chromosomes but there are fairly primitive 8-chromosome types. In both 10-chromosome and 8-chromosome series there is abundant evidence of progressive development from the woody-based perennial types with large simple leaves, few large heads, large florets, and large, unspecialized fruits to the short-lived annual forms with small or dissected leaves, numerous small heads, small florets, and very small or highly specialized fruits. The basic, primitive number is 10 and there are three 5-paired phylogenetic lines, one in each subgenus. There is also sufficient

evidence that the subgenera are not separated by fixed limits. From the peculiarities of certain species, such as *aurea* and *hypochaeridea* in *Catonia*, *patula*, *tingitana*, and *neglecta* in *Eucrepis*, and *albida* and *fontiana* in *Barkhausia*, it is clear that *Catonia* tends to merge into *Eucrepis* and the latter into *Barkhausia*. From such evidence it appears highly probable that the three 5-paired phylogenetic lines, one in each subgenus, had their origin in a common nexus. At any rate the genus must be looked upon as a natural unit.

9. *Chromosome morphology in Crepis*.—The chromosomes of *Crepis* species are of three distinct types, namely, those with a subterminal constriction, those with a subterminal constriction and bearing a satellite, and those with a median constriction. By comparing total length and relative length of the arms, chromosomes of the first general type are subdivided into classes known as A, B, and C. The satellite-bearing chromosome is called D and the small median-constricted chromosome, E. The only important exception to this general scheme is that in three subgroups under *Eucrepis* the large A chromosome has a median constriction.

10. *The basic genom*.—All the 5-paired species have one pair each of chromosome types A, B, C, D, E.

11. *Derived genoms and modes of derivation*.—All the 4-paired species have types A, B, C, D. The two 3-paired species have A, B or C, and D. The 6-paired species are variable. In *Catonia* they seem to have two pairs of A chromosomes, one or two pairs respectively of B or C types, and one pair of D's. In *Eucrepis* they have one, two, or three pairs of A type (sometimes with median constriction, sometimes with a satellite or compound), one, two, or no pairs of D type, and no, one, or two pairs of B, C, and E types. This evidence on composition of the 6-paired genoms indicates that these species were derived from 5-paired ancestors through hybridization, and that 12 is not a primitive number in *Crepis*. The 7-paired species have A, B, C, D, and E types with duplication of C, D, or E. This also suggests hybrid origin for these species and indicates that 14 is not a primitive number. All 8-paired species, so far as known, have only A, B, C, and D types and are polyploids. Analysis of distribution of chromosome types in the higher-numbered species has not been attempted, but dissimilarity of the satellite-bearing chromosomes in 11-paired American species adds sufficient proof of their origin through interspecific hybridization and amphidiploidy. Similar evidence is found in the two Old World octoploid species, *C. biennis* and *C. ciliata*.

12. *Chromosome morphology and phylogeny in Crepis*.—(a) Morphologically similar species have similar chromosomes. (b) Similarity in chromosome types and in details of size and shape is an index of phylogenetic relationship. (c) Both increase and decrease in chromosome size have occurred in the evolution of the genus. (d) There is a general ten-

dency toward reduction of size of chromosomes concurrently with reduction in size of the plant and reduction or specialization of organs. (e) There have been many changes in chromosome shape, as determined by relative length of the arms, and by these differences chromosomes of the same type from different species can be identified in hybrids. (f) This fact makes it possible, by analysis of the haploid genom, to determine the mode of origin of certain species.

13. *Chromosomes and taxonomy.*—Chromosome number and morphology is a taxonomic criterion of great value in this genus. But it must be used in connection with other available criteria such as comparative morphology and geographic distribution. Certainly, absolute identity of the chromosomes cannot be set up as of paramount importance in the classification of species, for specific entities are known in which the different forms exhibit differences in number, size, or shape of the chromosomes. The genus is still evolving and visible changes in the chromosomes are part of the process.

14. *Evolutionary processes in Crepis.*—(a) In view of the evidence here summarized that there is one most primitive chromosome number and type of genom in *Crepis*, it is clear that the primary evolutionary process which has operated in the history of the genus, as we now know it, is some mode of transformation by which 8- and 6-chromosome species have been derived from 10-chromosome ancestors. (b) Next in importance is interspecific hybridization and amphidiploidy. (c) Third comes polyploidy. (d) Superimposed upon and operating concurrently with the foregoing is gene mutation. (e) Origin of species with new chromosome numbers through transformation and through interspecific hybridization with amphidiploidy must have occurred early in the evolution of the genus. (f) All four processes have been at work during comparatively recent times.

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# MEIOSIS IN CERTAIN INTERSPECIFIC HYBRIDS IN CREPIS AND ITS BEARING ON TAXONOMIC RELATIONSHIP

BY

E. B. BABCOCK AND S. L. EMSWELLER

## CREPIS NICAENSIS Balb. $\times$ C. SETOSA Hall. f.

MOST CREPIS SPECIES are excellent subjects for cytological research because of their generally large and morphologically distinct chromosomes. The genus includes three subgenera, in each of which numerous representative species have been studied with reference to comparative morphology, geographic distribution, and chromosome relations (Babcock and Cameron, 1934; Babcock, 1936). Cytogenetic studies on several hybrids between species in the same subgenus and in different subgenera have exhibited phenomena that throw light on problems of taxonomy, phylogeny, and evolution (Babcock and Navashin, 1930). The present investigation was undertaken with the hope of contributing further evidence on some of these problems.

### SPECIES AND HYBRIDS INVESTIGATED

These investigations are concerned with hybrids between *Crepis nicaensis* Balb., of subgenus *Eucrepis*, and two subspecies of *Crepis setosa* Hall. f. of subgenus *Barkhausia*. Some of the  $F_2$  derivatives were also studied. Plants of the two subspecies of *C. setosa*, namely, *typica* (accession 2623) and *Topaliana* (accession 2671) were used as paternal parents, and a single *nicaensis* plant (accession 2700) furnished the maternal gametes. The diploid chromosome number of both species is 8.

The strain of *C. nicaensis* used in this study came from northern Italy. The species occurs sporadically in lower montane regions from the Eastern Pyrenees to Macedonia. Plants found in the wild state are tall, erect, and simple-stemmed with a few branches near the top bearing a few medium-sized flower heads. Under cultivation the plants are either annual or biennial; they retain the same upright habit as in the wild but are very vigorous, with long branches and many heads. As a rule *nicaensis* plants have a stronger central axis than do *setosa* plants and the basal or rosette leaves are very different, although there is noticeable variation in both species. In details of the heads, florets, and fruits the differences are even more marked. Differences in the achenes

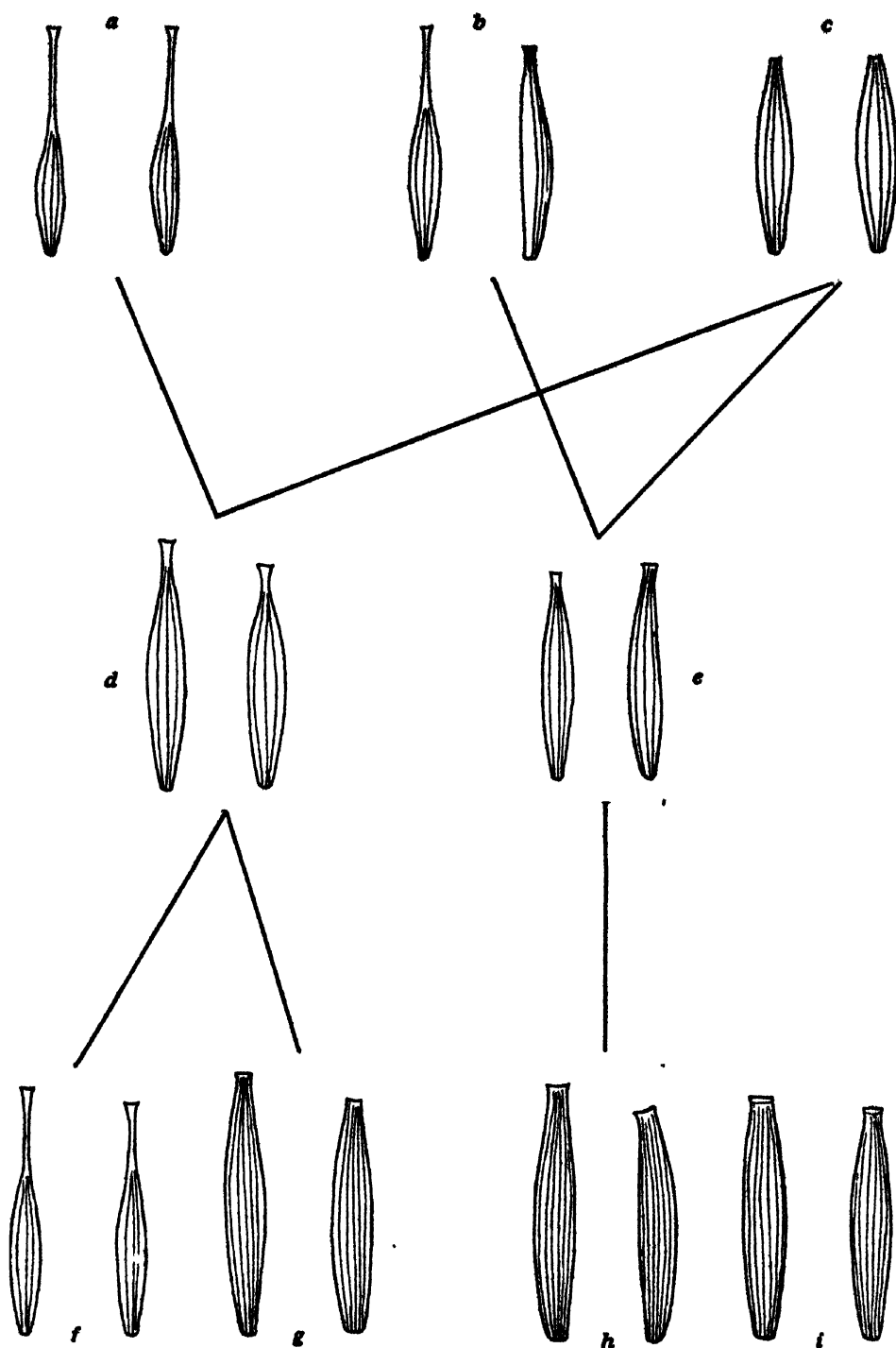


Fig. 1. Achenes of: *a*, *Crepis setosa typica* (2623); *b*, *C. setosa Topaliana* (2671); *c*, *C. nicaeensis* (2700); *d*, *C. nicaeensis* × *C. setosa typica* F<sub>1</sub>; *e*, *C. nicaeensis* × *C. setosa Topaliana* F<sub>1</sub>; *f*, an F<sub>1</sub> derivative with 8 chromosomes; *g*, *h*, F<sub>1</sub> derivatives with 24 chromosomes; *i*, *C. biennis*.

are of special interest in this study. In *nicaeensis* the achenes are golden brown, about 3 mm. long and 0.6 mm. wide, terete, narrowed at base and summit, with no beak or prolongation of the apex, and with ten broad, nearly smooth, longitudinal ribs (fig. 1c).

In *C. setosa*, considered as a whole, the plants are more slender, the habit more bushy, and the heads more numerous and smaller. Besides other distinguishing characters, the achenes are tawny, 3–5 mm. long and 0.3–0.6 mm. wide, and the body is terete, with ten ribs, narrowed at base and attenuate at summit into a slender beak equal to or shorter than the body (fig. 1a, b). The strain of *C. setosa typica* used in these investigations was obtained from a wild population in Savoy, France. This subspecies occurs rather commonly at low elevations from the eastern Pyrenees to Macedonia. Our accession of subsp. *Topaliana* came from a wild population in eastern Thessaly. This subspecies has a restricted distribution in northern Greece and a few forms which are intermediate between the two subspecies have been found in northwestern Thessaly and Epirus.

Some of the characters that distinguish the two subspecies are listed in table 1. Although these data were obtained from only two plants of one subspecies and one of the other, yet these plants were fairly typical of their respective entities. Plants of *typica* are normally larger and more robust than *Topaliana* plants. Differences in the basal and cauline leaves are shown in figure 2a, b. Certain differences in the achenes are discernible in figure 1a, b. In the *typica* strain the beak is equal in length to the body of the achene and all the achenes in a head are closely similar. In *Topaliana* the marginal achenes are notably different from the rest of the achenes in the head. They are somewhat compressed, paler in color, and merely attenuate, not finely beaked, as is shown by the right-hand achene in figure 1b, and the inner achenes have a relatively longer body and shorter beak than do those in the *typica* strain used. Another difference which, although minute, was consistent in each plant was found in the setae (bristles) borne on the involucre bracts and peduncle. In the *typica* strain these setae are slightly longer and more slender than those of the *Topaliana* strain. This is especially interesting because of the failure of setae to appear in the  $F_1$  (30.31) of which *typica* was a parent, whereas setae were abundant in the other  $F_1$  (30.32), as is shown in plate 1d, e. The first impression given by these facts is that the particular *typica* plant which was used as male parent of 30.31 must have been heterozygous for setae, whereas the *Topaliana* plant used in producing 30.32 was homozygous. It was shown by Collins (1924) that in *Crepis capillaris* glabrous involucre it inherited as a simple recessive to pubescent involucre. But here the pubescence consists of hairs, not setae. Furthermore, plants with glabrous involucre have never been reported

in *C. setosa*; on the contrary, these peculiar setae seem to be a constant specific character in this species. Certainly no plants with glabrous involucre were observed among several strains of *typica* while they were under cultivation. This fact, together with the structural difference in the setae of the two subspecies noted above, may be indicative of a

TABLE 1

DIFFERENTIATING CHARACTERS IN THE SUBSPECIES OF *Crepis setosa*

| <i>2623. Typica</i>  | <i>2671. Topaliana</i>  |
|--|---|
| Large, robust  | Small, slender  |
| Height (2 plants), 65-75 cm.   | Height (1 plant), 38 cm.  |
| Stem erect with several long branches from base, each with a secondary branch from each node; secondary branches erect, forming a narrow angle with main branch  | Stem erect with a branch from each node, but branches shorter than axis and spreading at a wide angle from it   |
| Involucre setose, the bristles slightly longer and more slender than in 2671. Bristles of same type also rather plentiful on peduncle  | Involucre setose, the bristles shorter and slightly thicker. Bristles on peduncle very few and diminished   |
| Achenes all similar, or the marginal ones parthenocarpic, but even then usually beaked and colored like the inner ones; 3-4 mm. long, deep tawny, the body oblong, subterete, strongly 10-ribbed, beak equal to body, conspicuously expanded to form a white inverted cone below the broad pappus disk | Achenes of two shapes, 3-4 mm. long; the marginal ones very pale or whitish on ventral face, long-fusiform, strongly attenuate toward summit or shortly beaked, obscurely ribbed; inner achenes pale tawny, the body fusiform, subterete, delicately 10-ribbed, beak a little shorter than body, not expanded below pappus disk |
| Style branches bright green  | Style branches pale greenish yellow   |

genetic difference between the subspecies with respect to setae. Attempts were made to obtain hybrids between these two subspecies of *C. setosa*, but they failed, so there is no further evidence bearing on this particular question.

Hybrids between *C. nicaeensis* and *C. setosa* were obtained with difficulty. Only four  $F_1$  plants were produced, two of each with *typica* and *Topaliana* as male parent. For brevity's sake the two crosses will be referred to hereafter as hybrid A and hybrid B. The  $F_1$  hybrid sibs were very similar, but there were consistent differences between the two  $F_1$

families. Some of these differences are shown in table 2. The leaf types of the parents and  $F_1$  hybrids are shown in figure 2. Not much significance can be attached to these differences because the parents, like those in other species of *Crepis*, are rather variable in leaf shape; but, taken along with the other differences in the hybrids, these leaf differences may also indicate genetic diversity between the subspecies. Certain differences in anthocyanin in the  $F_1$  plants may have been due to heterozygosity in the *nicaeensis* parent. One plant from each cross was pressed

TABLE 2

DIFFERENTIATING CHARACTERS IN HYBRIDS A AND B (2 PLANTS OF EACH)

| A. <i>C. nicaeensis</i> ♀ × <i>C. setosa typica</i> ♂          | B. <i>C. nicaeensis</i> ♀ × <i>C. setosa Topaliana</i> ♂       |
|--|--|
| Achenes average 4.27 mm. long; ribs as in <i>C. nicaeensis</i> | Achenes average 3.47 mm. long; ribs similar but less prominent |
| Involucres and peduncles glabrous                              | Involucres and peduncles setose                                |
| Anthocyanin restricted to base of plant                        | Anthocyanin present in upper part of plant                     |
| Basal and cauline leaves lacinate                              | Basal leaves coarsely dentate; cauline leaves nearly entire    |

for the herbarium; the others were used in the present study. The two hybrids differed notably in fertility. Hybrid A produced 545 open-pollinated achenes and hybrid B, only 120.

From hybrid A one hundred  $F_2$  achenes were sown. They produced ninety-three seedlings. Three of these died at the cotyledon stage and three more after they had developed a small rosette of basal leaves (see plate 2*h*, *i*). The chromosomes of the six seedlings that died were not examined. The remaining plants were grown to the large rosette stage. Chromosome numbers were determined from somatic plates in root tips. Only three had 8 chromosomes; the remaining eighty-four all had 24 chromosomes.

From hybrid B twenty-five achenes were sown, and nineteen seedlings were obtained. Two of these died in the cotyledon stage. The other seventeen were grown to maturity; of these, two had 8 chromosomes and fifteen had 24 chromosomes.

The 24-chromosome  $F_2$  derivatives must have resulted from natural crossing with *Crepis biennis* ( $n=20$ ). Plants of this species were in flower along with the  $F_1$  hybrids, which were purposely left unprotected. The leaves of the 24-chromosome derivatives were more or less like those

of *C. biennis*. This is not necessarily significant, because the leaves of *nicaeensis* also resemble those of *biennis*; but when these leaves are compared with those of their 8-chromosome sibs, the difference is striking



Fig. 2. Basal and cauline leaves from: a, *Crepis setosa typica*; b, *C. setosa Topaliana*; c, *C. nicaeensis*; d, *C. nicaeensis* ♀ × *C. setosa typica* ♂ × F<sub>1</sub>; e, *C. nicaeensis* ♀ × *C. setosa Topaliana* ♂ × F<sub>1</sub>.

because the leaves of the latter strongly resembled those of *setosa* (see plate 15f, g), whereas the leaves of the 24-chromosome plants were very like those of *biennis* (plate 15a-e). Fortunately these 24-chromosome derivatives were slightly fertile, and the achenes were like those of *bien-*

was in size and shape (see fig. 1) and in the higher number of ribs which is characteristic of *biennis*.

The five 8-chromosome  $F_2$  derivatives were closely similar in general appearance and very precocious in development as compared with the 24-chromosome sibs. Two of them are shown in rosette stage in plate 15f, g. The one on the right is three weeks older than the other. Two of the 8-chromosome derivatives died from an unknown cause when the flower stalk was a few inches high, and another was attacked by a species of *Botrytis* just before flowering. The two plants that produced flowers were from hybrid A. Their fertility was low, one producing twelve achenes and the other, seven.

This paper is chiefly concerned with a study of meiosis in the  $F_1$  hybrids and the two 8-chromosome  $F_2$  derivatives that produced flowers.

#### CYTOLOGICAL METHODS

Root tips were fixed in chromacetic formalin and stained with Haidenhain's iron haematoxylin, as described by Hollingshead and Babcock (1930). At first the buds were fixed in the Carnoy-Navashin solution, dehydrated in the alcohols, cleared in xylol, and imbedded in paraffin. Buds treated in this manner were usually cut with difficulty because of hardening in the higher alcohols. To remedy this situation, a butyl alcohol series was substituted as a clearing agent in place of the regular xylol series. In this method the buds were run up to 80 per cent alcohol, then through the following series of alcohols (Zirkle, 1931) :

- (a) Water 15 cc., absolute ethyl alcohol 50 cc., butyl alcohol 35 cc., 2 to 3 hours.
- (b) Water 5 cc., absolute ethyl alcohol 40 cc., butyl alcohol 55 cc., 2 to 3 hours.
- (c) Absolute ethyl alcohol 25 cc., butyl alcohol 75 cc., 2 to 3 hours.
- (d) Butyl alcohol. At least 2 changes, leaving in the 2d change at least 12 hours.

Infiltration was accomplished by pouring melted paraffin into a vial, allowing it to cool, and then pouring over it butyl alcohol containing the buds. As few as four changes in the oven over a period of two days were found to be sufficient to remove all traces of butyl alcohol; the buds treated in this manner were soft, easily cut, and stained readily in both gentian violet and Haidenhain's iron-alum haematoxylin.

In order to concentrate more material on a slide, imbedded buds were remelted in the oven, and from two to four were mounted on one block. Melted paraffin was slowly dropped over the buds and cooled rapidly, and the block was finally trimmed for cutting. Both cross and longitudinal sections were cut. The latter proved to be most useful, because a large number of PMC's could be studied in the same locule, and because irregular stages found in  $F_2$  plants were more easily studied when ad-



jacent to "normal" cells. All slides from each block were kept separate, and a slide containing material from near the center of the buds was stained to determine whether the desired meiotic figures were present; if so, the remaining slides of the series were stained.

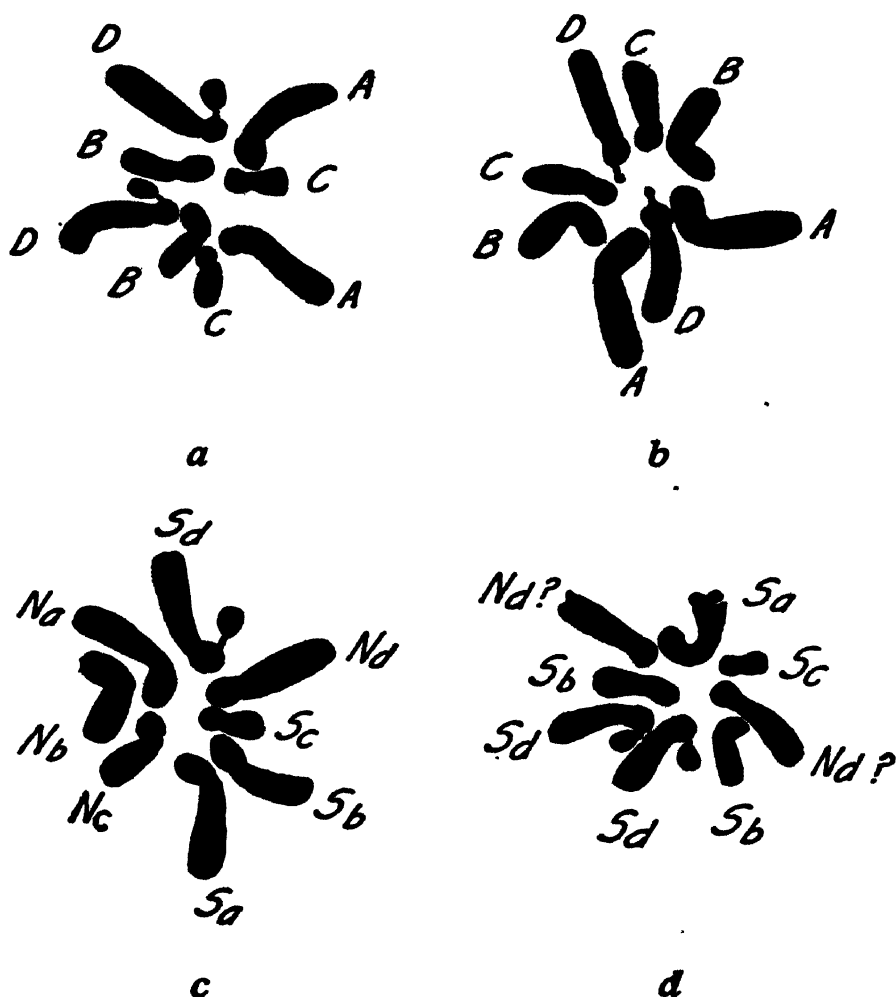


Fig. 3. Chromosomes at mitotic metaphase in root-tip cells of: a, *Crepis setosa*; b, *C. nicaeensis*; c, *C. nicaeensis*  $\times$  *C. setosa*  $F_1$ ; d, an 8-chromosome  $F_2$  derivative.  $\times 2500$ .

Figure 3 and figure 4c were drawn at a magnification of 2500 diameters and were reproduced without reduction. Figures 4a and 4b were drawn at a magnification of 4000 diameters and reproduced without reduction. Figures 5e and 6c were drawn at a magnification of 1800 diameters. All other drawings were made at a magnification of 2500 diameters and were reduced one-fourth in reproduction.

## SOMATIC CHROMOSOMES

Somatic metaphase chromosomes of *Crepis setosa* are shown in figure 3a; those of *C. nicaeensis* in figure 3b. As the chromosomes of the two *setosa* subspecies were apparently identical, only one plate is shown. According to Navashin's scheme of chromosome designation, both species have one pair each of types A, B, C, and D. These designations are not

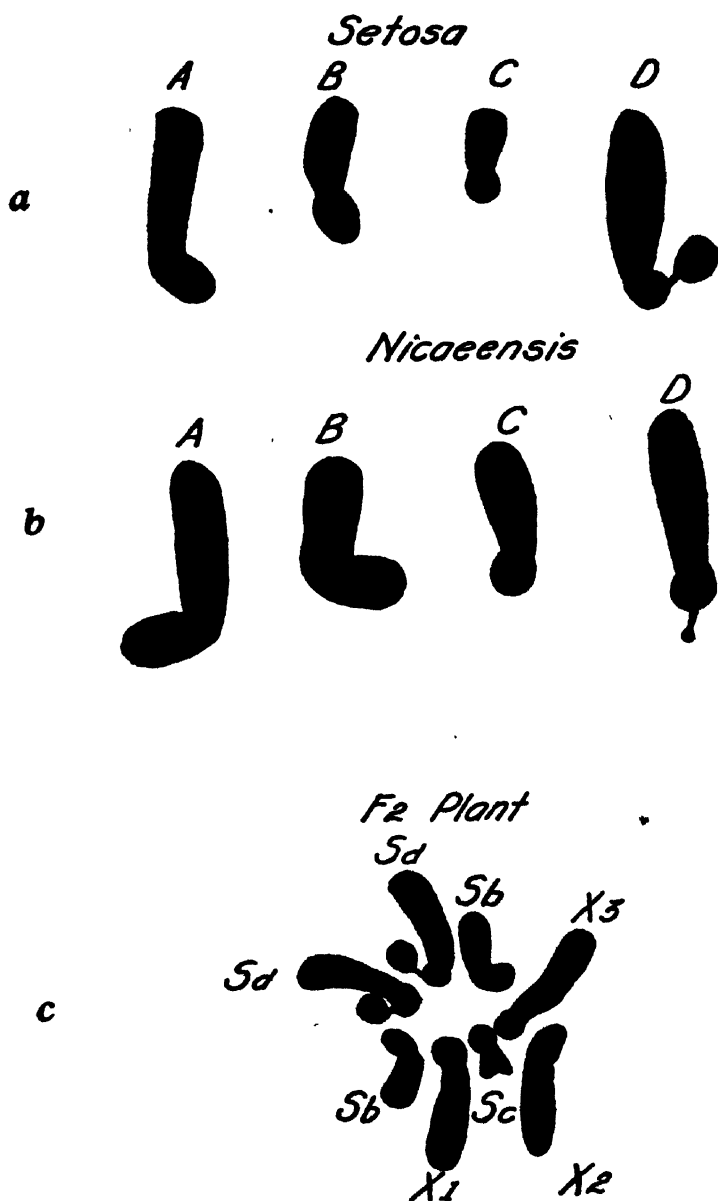


Fig. 4. a, b, Haploid chromosomes of *Crepis setosa* and *C. nicaeensis*  $\times 4000$ . c, chromosomes at mitotic metaphase in a root-tip cell of an 8-chromosome  $F_2$  plant showing three doubtful members,  $X_1$ ,  $X_2$ ,  $X_3$ .  $\times 2500$ .

intended to imply homology but merely general similarity in size and shape. There is difference enough in chromosome morphology to make identification of each set in  $F_1$  a relatively simple matter (fig. 3c). Nava-shin (1928) reported loss of the satellite of the *tectorum* D chromosome in the  $F_1$  *capillaris-tectorum* hybrid. This observation was confirmed by Hollingshead in the same hybrid (1930). In the  $F_1$  *nicaeensis-setosa* hybrid the *nicaeensis* D chromosome is similarly modified and careful measurements indicated an increase in size of the proximal arm. This change makes it more difficult to distinguish between the B chromosome of *setosa* and the modified D chromosome of *nicaeensis*. The chromosomes of the five  $F_2$  plants discussed in this paper are shown in figure 3d. In order to check the camera-lucida drawings, photomicrographs were made from normal somatic plates (plate 14f). The chromosomes appear to be as follows: from *setosa*, one A, two B, one C, and two D; and from *nicaeensis*, two modified D chromosomes. This genom, however, is outside of normal expectations and studies of meiotic behavior in both  $F_1$  and  $F_2$  indicate that one of the two chromosomes, which is morphologically similar to the *nicaeensis* D chromosome, is perhaps a modified *setosa* B chromosome. The evidence favorable to this hypothesis will be discussed under meiosis.

It is unusual that all five of these plants should have the same chromosome complement, and that no other combination of eight chromosomes was found. The eight  $F_2$  plants that died in the seedling and very early rosette stages were most likely 8-chromosome types that were unable to survive. When the first lot of  $F_2$  seed was planted, some care was exercised to eliminate the shrunken achenes. When a second planting was made, the achenes were carefully graded into large and small lots. There was a mortality of 40 per cent in the seedlings from the small achenes and of only 2 per cent in seedlings from large achenes.

#### MEIOSIS IN $F_1$

##### Hybrid A (*C. nicaeensis* ♀ × *C. setosa typica* ♂)

This hybrid exhibited rather high regularity in its meiotic behavior. In figure 5 the drawings are from several slides, all stained with Haidenhain's, with the exception of *b*, *d*, and *h* which were stained in gentian violet. The number of bivalents ranged from one to four. Figure 5a-d shows associations of  $4_{II}$ ,  $3_{II} + 2_I$ ,  $2_{II} + 4_I$ , and  $1_{II} + 6_I$ , respectively. The chromosomes in *b* and *c* are drawn out of position for the sake of clarity. Figure 5e shows four adjacent PMC's in the same locule, all exhibiting complete pairing. In three a small chromosome is loosely paired with a large one. The small chromosome is undoubtedly the *setosa* C chromosome. Figure 5f shows  $3_{II} + 2_I$  at IM. The univalents were apparently

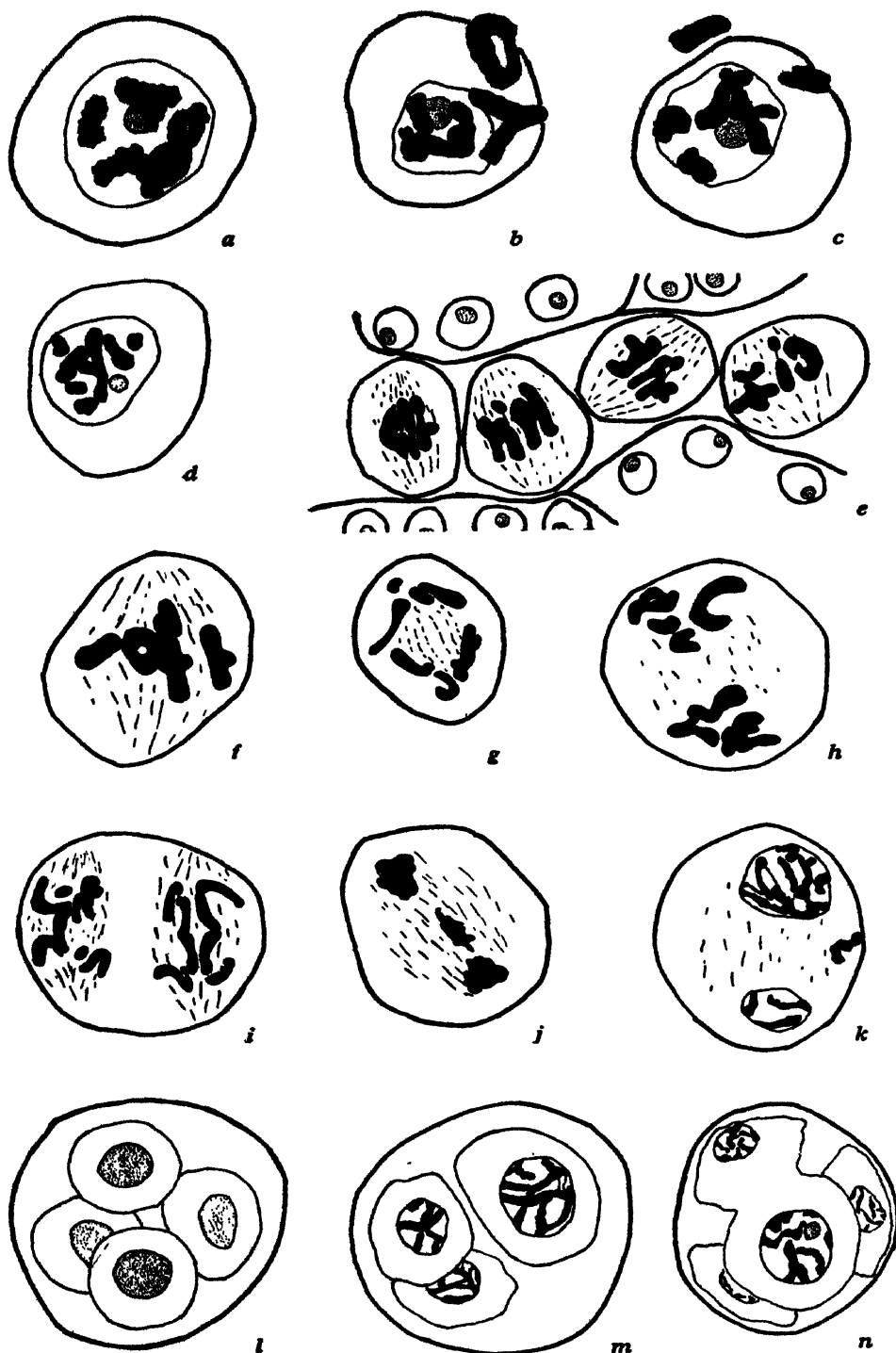


Fig. 5. Meiosis in hybrid A (*C. nicaeensis*  $\times$  *C. setosa typica*). a-d, selected diakinesis figures showing variable pairing (in b and c certain chromosomes have been drawn outside the nucleus for clarity). e, adjacent PMC's showing complete pairing. f, IM— $3_{II} + 2_I$ . g, IA. h, IIM. i, IIA. j, k, m, n, types of irregularities. l, "normal" tetrad.  $\times 1875$ , except e, 1575.

not arranged on the spindle but were placed to the side of it. Their size indicates that neither is the *setosa* C chromosome. In ten cells showing  $3_{II} + 2_I$ , the C chromosome of *setosa* was one of the two unpaired chromosomes in only four cells.

Figure 5*g* shows a IA in which the *setosa* C chromosome is easily recognized. The chromosomes have not yet assumed their characteristic shapes, as is also true in the IIM shown at *h*, which may have followed such an anaphase. Figure 5*i* is an excellent IIA, showing the chromosomes in a nearly normal somatic condition. In the group to the right, the D chromosome of *setosa* can be recognized as well as its small C chromosome in the group at the left. Such a IIA would probably give rise to a tetrad similar to figure 5*l*. Very few irregularities were observed. Figure 5*j* shows a IT in which a portion of the chromatin has failed to reach either pole and will probably be left in the cytoplasm as a micronucleus. In figure 5*k* unequal nuclei are seen, with a chromatic body in the cytoplasm at the right. There was no trace of a membrane about this chromatic body. Possibly figure 5*m* represents a triad with a diploid and two haploid nuclei. It was the only example of this type found in the material examined. A type of tetrad irregularity that occurred very rarely is shown in figure 5*n*. There has been an unequal division of chromatin in the IIM of one nucleus of the dyad, whereas the other nucleus appears to have divided more regularly.

The frequencies of pairing in the two  $F_1$  plants and in the  $F_2$  plants are shown in table 3.

The large number of instances in which four bivalents occur is rather remarkable for *Crepis* hybrids involving different subgenera. The pairing is also rather close, and there is apparently a very intimate association of the chromosomes of most bivalents. The small C chromosome of *setosa* can frequently be identified, and it is usually very loosely paired with a *nicaeensis* chromosome. The generally close association of these chromosomes from widely separated species is quite different from the looseness of pairing reported in most of the other interspecific hybrids of *Crepis*. Avery (1930) published meiotic figures of hybrids involving *Crepis leontodontoides* with five other species—*tectorum*, *capillaris*, *parviflora*, *Marschallii*, and *aurea*. In many cells the bivalents are composed of rather loosely associated chromosomes which typically form elongated bivalents. The same situation also occurs generally in the hybrids studied by Babcock and J. Clausen (1929), who also published meiotic figures of *Crepis aspera* and *C. bursifolia*. The bivalents formed in these species show an association more nearly resembling the bivalents in our hybrid. Such close association in this hybrid indicates at least that conditions were rather favorable for exchange of material between chromosomes of the two species. This supposition is strengthened by the investi-

gation of meiosis in the hybrid *Crepis divaricata*  $\times$  *C. Dioscoridis* by Müntzing (1934). Although the amount of pairing was noticeably lower than in our hybrids, the nature of the association was similar. Cross-shaped bivalents sometimes occurred, which would indicate interstitial chiasmata, and "the frequent occurrence of chromatin bridges and fragments at IA is too regular to be due simply to non-homologous association."

TABLE 3  
BIVALENT FORMATION AND ACHENE PRODUCTION IN  $F_1$  AND  $F_2$

|                | 4 <sub>II</sub> | 3 <sub>II</sub> 2 <sub>I</sub> | 2 <sub>II</sub> 4 <sub>I</sub> | 1 <sub>II</sub> 6 <sub>I</sub> | 0 <sub>II</sub> 8 <sub>I</sub> | Per cent irregularities | Per cent "good" pollen | Number of achenes |
|----------------|-----------------|--------------------------------|--------------------------------|--------------------------------|--------------------------------|-------------------------|------------------------|-------------------|
| Hybrid A.      | 122             | 10                             | 1                              | 1                              | 0                              | 9                       | 92                     | 545               |
| Hybrid B.      | 64              | 32                             | 16                             | 2                              | 0                              | 43                      | 58                     | 120               |
| $F_2$ no. 1... | 22              | 44                             | 2                              | 6                              | 1                              | 71                      | 48                     | 7                 |
| $F_2$ no. 2... | 31              | 72                             | 7                              | 12                             | 2                              | 75                      | 42                     | 12                |

Hybrid B (*C. nicaeensis* ♀  $\times$  *C. setosa Topaliana* ♂)

In this hybrid the meiotic behavior of the chromosomes is considerably more irregular than in hybrid A. As shown in table 3, these irregularities comprise 43 per cent of the total, as compared with about 9 per cent for the same classes in hybrid A. In hybrid B, as well as in hybrid A, no cells were found with a complete failure of bivalent formation.

A diakinesis figure with 4<sub>II</sub> is shown in figure 6a. In figure 6b there are 3<sub>II</sub> + 2<sub>I</sub>; and the smallest univalent is very probably the C chromosome of *setosa*. Figure 6c represents three adjacent pollen mother cells with 4<sub>II</sub>, 3<sub>II</sub> + 2<sub>I</sub>, and 2<sub>II</sub> + 4<sub>I</sub> and shows the occurrence of loosely paired bivalents and the close association of variable pairing in the same locule. This figure may be compared with figure 5c, to show the looser type of pairing and relatively less frequent formation of 4<sub>II</sub> in this hybrid than in hybrid A. In figure 6d the association of the small *setosa* C chromosome with a large *nicæensis* chromosome forms a bivalent somewhat similar to one formed in hybrid A. One of the two cells observed, in which but one bivalent was formed, is shown in figure 6e in which the chromosomes were very loosely arranged near the equatorial plate. Figure 6f, g represent adjacent PMC's, one of which shows 2<sub>II</sub> + 4<sub>I</sub> and the other 3<sub>II</sub> + 2<sub>I</sub>. In the second cell, two of the bivalents are very loosely associated, in striking contrast to the same situation in hybrid A (fig. 5f). In figure 6h one chromosome is seen lagging at the equator, four are moving to one pole, and three are going to the other. Three of the disjoined chromosomes already show equational splitting for the homoeotypic division. In figure 6i one chromosome is at one pole, five are at the

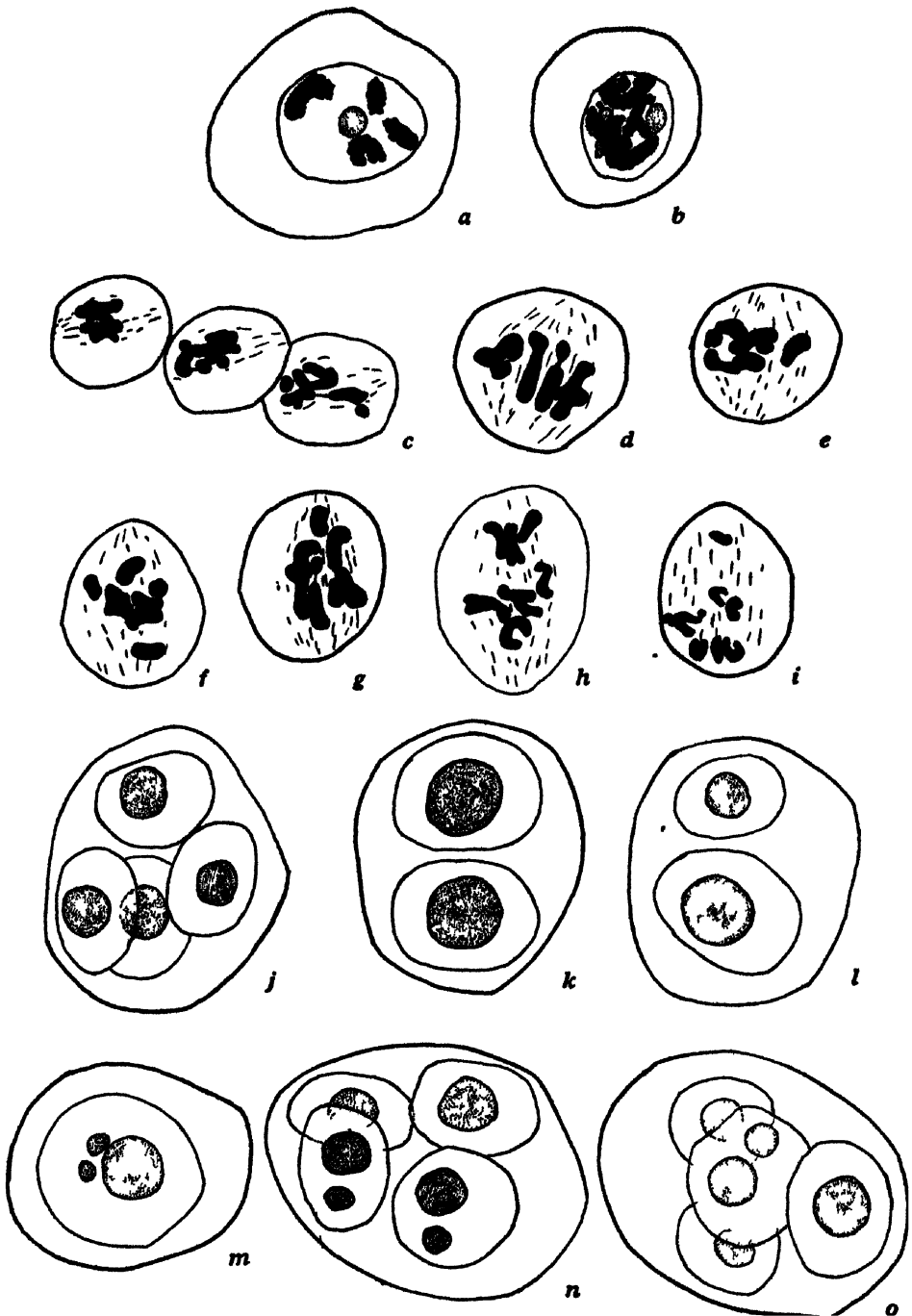


FIG. 6. Meiosis in hybrid B (*C. nicotiana* × *C. setosa* Topaliana). a, b, diakinesis. c, adjacent PMC's showing variable pairing. d-g, metaphase plates showing rather loosely formed bivalents h, i, IA showing unequal distribution of chromosomes. j, normal tetrad. k, dyad l-o, variations found in same locules with "normal" types. × 1875, except c, 1575.

other pole, and two are lagging at the equator but appear to have begun migrating toward the pole with the larger chromosome number. An apparently normal tetrad is shown in figure 6j. Figure 6k represents a dyad in which the nuclei appear to be equal in size. Very few irregularities of this sort were seen in this plant, and where they did occur they were in locules in which tetrads occurred. Figure 6l shows a dyad in which the nuclei are unequal in size. This condition could have followed an unequal distribution of chromosomes like that shown in *h*. A normal monad was not found in this plant, but several similar to figure 6m were seen. Most of the tetrads were abnormal, micronuclei being very common.

### MEIOSIS IN $F_2$

The various stages in meiosis of the  $F_2$  plants with 8 chromosomes are shown in figure 7. These drawings were made from smear preparations from the two plants which flowered. Diakinesis figures with  $4_{II}$  and  $3_{II} + 2_I$  are shown in figure 7a, b. Some IM figures are shown in figure 7c-j depicting  $4_{II}$ ,  $3_{II} + 2_I$ ,  $1_{II} + 6_I$ , and  $8_I$ . In figure 7i the C chromosome of *setosa* is a member of the only bivalent formed. Two IA groups are seen in figure 7k, l. In figure 7l some of the separated chromosomes are splitting for the homoeotypic division. An irregular assortment of 5 and 3 chromosomes is seen in figure 7m. A division of this type could give rise to very small pollen grains like those shown in figure 8i. The chromosome group in figure 7n is interesting because it suggests a possible method of origin for polyploid gametes. The seven units at one pole may have occurred by nonconjunction in three pairs of chromosomes. This situation was observed but once and cannot account for the high frequency of large pollen found in this plant. Many of the tetrads were normal (fig. 7o), but usually irregular types were found (fig. 7p and fig. 8f, g, h).

The frequency of the irregularities in meiosis in these two plants is in rather close agreement, as is shown in table 3. It is very unusual that in so many cells only one or two bivalents are formed. Unfortunately meiosis in the two subspecies has not been studied, so it is not known whether either of them shows an appreciable amount of nonconjunction.

Chromosome morphology would seem to indicate that these  $F_2$  plants had six *setosa* chromosomes, one A, two B, one C, and two D, along with modified D chromosomes from *nicaeensis*. This complement, however, could be produced only if the *nicaeensis* D chromosome had paired part of the time with *setosa* C and part of the time with *setosa* A. This behavior would necessitate that the *nicaeensis* D chromosome have sectors, one homologous to a sector in the *setosa* C chromosome and another to a sector in the *setosa* A chromosome. If this were true, we should occasion-



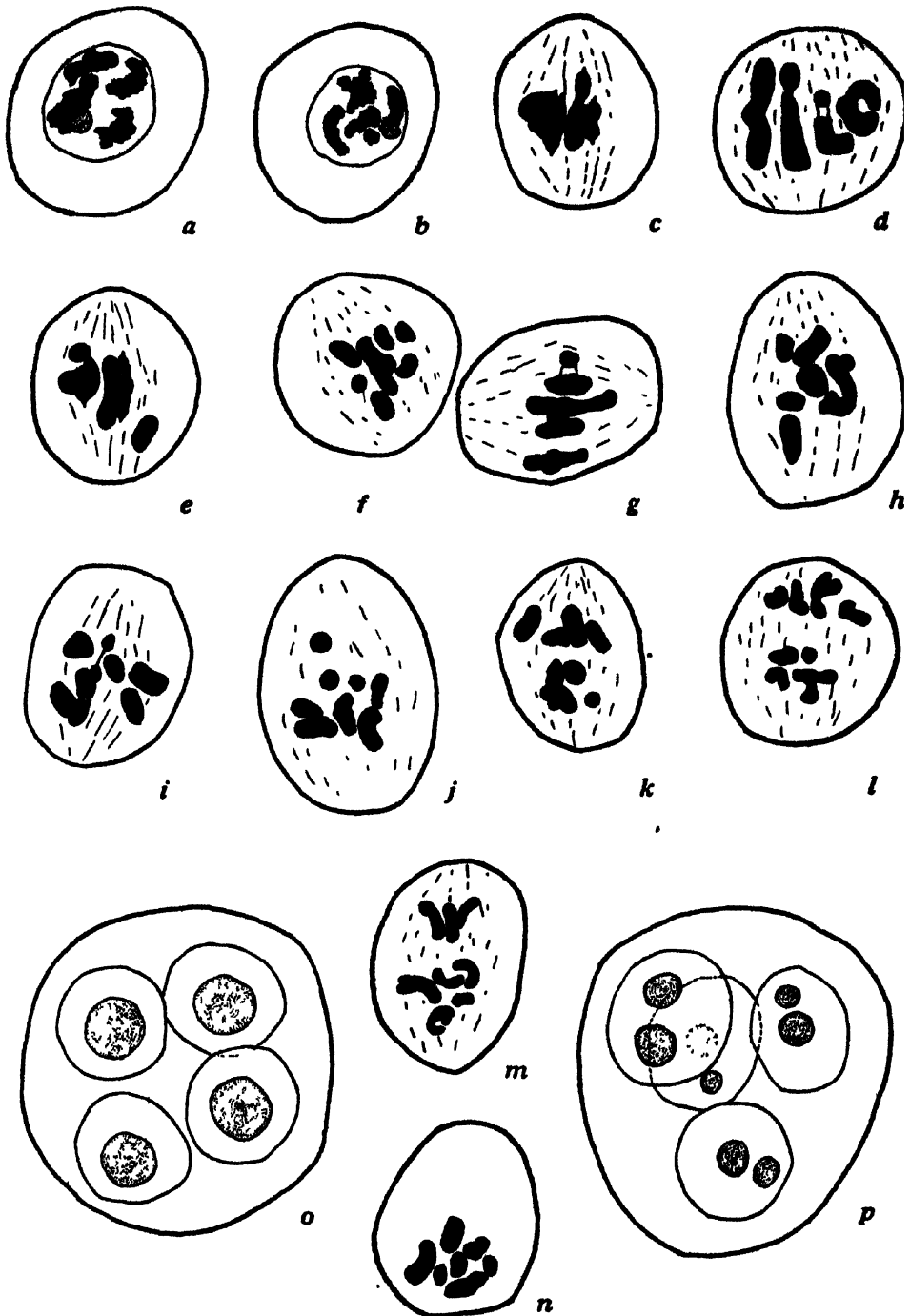


Fig. 7. Meiosis in 8-chromosome  $F_1$  plants. *a, b*, diakinesis showing  $4_{II}$  and  $3_{II} + 2_I$ ; in *b* note the small unpaired chromosome (see text). *c-j*, IM showing variation in number of bivalents. *k-m*, IA (note small C chromosome of *setosa* in *l* and unequal distribution of chromosomes in *m*). *n*, seven chromatid bodies at one pole; possibly a result of attachment. *o, p*, "normal" and abnormal tetrads.  $\times 1875$ .

ally find trivalents formed in these plants. As trivalents are not found this view is apparently not tenable.

The interpretation which seems most logical is that one of the two chromosomes which appear like *nicaeensis* D chromosomes is in reality a new chromosome resulting from crossing over in  $F_1$ . Although five  $F_2$  plants had eight chromosomes that were apparently of the types described above, only two flowered normally and both were descendants of hybrid A. The pairing in this  $F_1$  hybrid was exceedingly regular, and bivalent associations were in most cells very intimate. Hence exchange of material probably took place in this hybrid. If pairing occurred between *setosa* A and *nicaeensis* C, and crossing over took place at or near the constriction of *setosa* A, a new chromosome would be produced, the major portion of which would be *setosa* A, and the presence of the proximal arm of the *nicaeensis* C chromosome would produce an element resembling a *nicaeensis* D chromosome. On this basis the complement of these  $F_2$  plants may consist of the following *setosa* chromosomes: one A, two B, one C, two D, and one modified A, together with one modified *nicaeensis* D chromosome. The haploid sets of *setosa* and *nicaeensis*, together with an  $F_2$  somatic plate, are shown in figure 4. In the  $F_2$  plant the chromosomes in question are marked  $X_1$ ,  $X_2$ , and  $X_3$ .

The chromosomes of these  $F_2$  plants may perhaps represent recombination products from crossing over in  $F_1$ . Although the chromosomes of each species are distinctively different in morphology, it would become exceedingly difficult to identify many possible new recombinations. Plants with other assortments of 8 chromosomes were unfortunately not found; they might have thrown considerable light on the question at issue. Navashin (1926) reported the appearance in *Crepis* of large atypical chromosomes, a phenomenon to which he gave the name "novation." Their occurrence was thought to result not from end-to-end fusion, but perhaps from translocation. Avery (1930) suggested that the variable pairing observed in the *Crepis* hybrids which she investigated "is a reflection of the transformational processes presumably responsible for the differentiation of the specific genomes." In a recent paper Navashin (1934) reports several occurrences of "sporadic amphiplasty" in which morphologically changed chromosomes were observed in the progeny of species hybrids. Poole (1932) has found cytological evidence of new chromosome types in derivatives of his *rubra-foetida* amphidiploid. In the hybrid, *Crepis divaricata*  $\times$  *C. Dioscoridis*, Müntzing (1934) observed the occurrence at IA of chromatin bridges and fragments and concluded that the simultaneous production of long chromatids with two spindle-fiber attachments and fragments without attachment is probably caused by crossing over between homologous segments in chromosomes from the two species. He emphasizes the point that "an unlim-

*ited number of new chromosome types may arise by the mechanism at work in this hybrid (or a number only limited by the number of chromomeres)."*

#### ORIGIN OF POLYPLOID GAMETES

When tetrad stages and pollen were studied in these  $F_2$  plants, it was found that a comparatively large number of monads and dyads were formed and that the pollen grains could be sorted into four rather definite size groups, as shown in figure 8j-l. The group listed as "small" (table 4) and shown in figure 8j is probably of the "normal" size. On the basis of measurements of pollen grains in this group these grains were equivalent to "good" pollen in the parent species.

TABLE 4  
SIZE OF POLLEN IN  $F_2$

| Giant | Large | Small | Very small |
|-------|-------|-------|------------|
| 35    | 78    | 115   | 16         |

In meiosis in  $F_1$ , and normally in  $F_2$ , whole chromosomes that were paired at IM separated and moved to the poles; and univalents were assorted at random without splitting. In the  $F_2$  plants, IA figures were finally found in which splitting of both disjoined and univalent chromosomes occurred. Figures 8a and 8b indicate that the splitting has taken place slightly earlier in the disjoined chromosomes. In both chromosomes there is no doubt that the split had been completed in early anaphase and that the resulting nuclei would have had 8 chromosomes each. This type of behavior will probably result in a binucleate condition like that shown in figure 8f. The complexity of the chromosome situation in this  $F_2$  plant makes it extremely probable that the resulting nuclei are very rarely alike qualitatively, even though each has 8 chromosomes. As the equational split has already occurred, it is unlikely that a second division of the chromosomes will occur; and various types of "diploid" gametes should result. Figure 8k is probably a "diploid" pollen grain resulting from such a division.

A second irregularity in meiosis was found which probably gives rise directly to the giant pollen grains that occur in these  $F_2$  plants. Here, spindle formation at IM is very weak or entirely lacking. The chromosomes arrange themselves on the equatorial plate and some bivalents are probably formed, although this point is not certain and is not essential to what follows. Each of the 8 chromosomes splits equationally, but there is no separation of the halves; a nucleus of 16 chromosomes is the result. The 16 chromosomes can be counted readily in figure 8e. Various

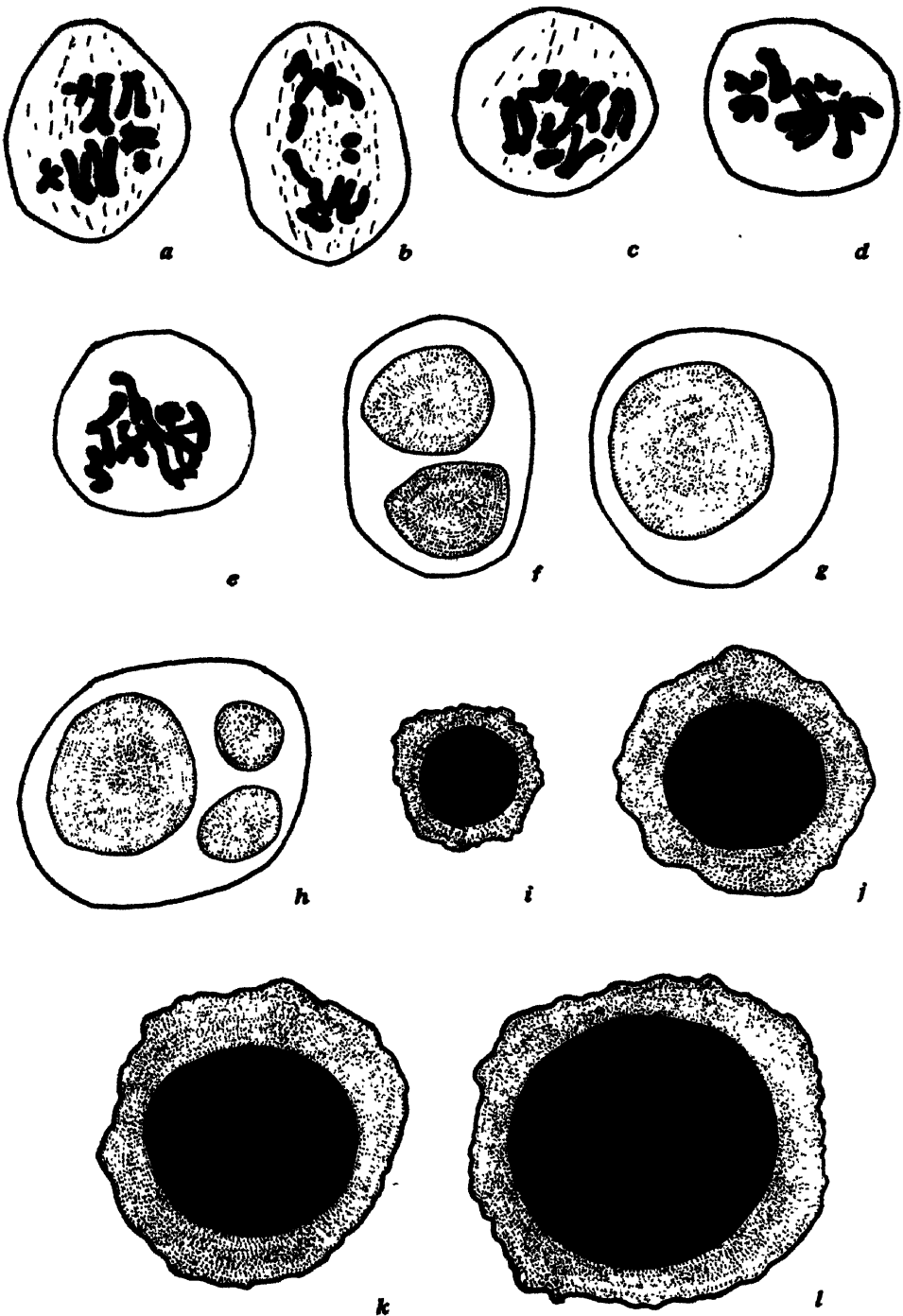


Fig. 8. Origin of polyploid gametes in an 8-chromosome  $F_1$  plant. *a, b*, first anaphase showing precocious splitting of chromosomes which will probably result in "diploid" nuclei. *c-e*, origin of 16-chromosome gametes through splitting of the 8 univalents in equatorial region with no succeeding division. *f, g, h*, "dyad," monad, and "triad." *i-l*, pollen grains of four size classes, all  $\times 1875$ .

stages of this type of division are shown in figure 8c, d, e. This condition would result directly in a monad (fig. 8g) which would develop into a giant pollen grain (fig. 8l).

Investigators working with widely scattered genera have reported the occurrence of giant pollen grains in many plants. De Mol (1923) found in Hyacinth, which had been subjected to abnormal conditions, large pollen grains in the nucleus of which was the diploid number of chromosomes. He interpreted their origin as due to an ineffective karyokinesis in the microspore rather than to any irregularities in the first or second division of the pollen mother cell.

Sakamura and Stow (1926) observed marked variation in the size of pollen grains in *Gagea lutea* from plants exposed to high temperatures. Cytological studies revealed several irregularities which they thought caused the abnormal pollen grains. In the first division they occasionally found an irregular distribution of chromosomes that resulted in pollen grains with varying chromosome numbers. Before the second division in some cells they observed that all chromosomes of the primary sporocyte gathered at the center of the cell and reconstructed a new nucleus without a cell division. They suggested that nuclei of this type probably gave rise to diploid pollen grains if a regular second division took place, or that, if this division were omitted, giant tetrad pollen grains would be formed. Second divisions were not observed.

Rosenberg (1926-27) investigated meiosis in a number of parthenogenetic species of *Hieracium*. He found a graded series of degeneration of meiosis ranging from the *boreale* type, in which a variable number of doubles and singles occur, to the *laevigatum* type, in which there is no conjugation whatever, namely, "semiheterotypic division." The latter type is often interrupted by a premature homoeotypic division whereby a "restitution nucleus" is formed. A new nuclear wall encloses the entire spindle figure and the chromosomes of this single large nucleus divide normally, producing pollen grains with the diploid number of chromosomes.

Karpechenko (1927), in tracing the origin of polyploid  $F_2$  plants from his generic hybrid *Brassica*  $\times$  *Raphanus*, found diploid pollen grains formed in the  $F_1$  in the following manner. At IM pairing between the chromosomes of each haploid set did not occur and spindle formation was very weak with no distribution of univalents. The complete omission of the heterotypic division was followed by a normal homoeotypic division, each chromosome splitting equationally and separating to the poles. This resulted in diploid dyads. A second splitting of some univalents was occasionally observed. Tetraploid gametes were found to originate as follows: "In the cells of the archesporium, at the division preceding reduction division, a division of the nucleus sometimes occurs

without formation of a new cell wall. As a result there are produced pollen mother cells with two nuclei, each of them containing 18 chromosomes. The chromosomes in both nuclei remain nonconjugating; at the first division both spindles fuse so that the anaphase shows all the univalents which later on form one nucleus containing all the chromosomes. A splitting of all the chromosomes then ensues, and in the majority of cases dyads arise with about 36 chromosomes in each cell."

The two  $F_2$  plants in which the polyploid gametes were observed were grown under separate environmental conditions. After the chromosome complements were determined, one plant was removed from Davis to Palo Alto and isolated. The second plant was isolated at Davis. Although Palo Alto and Davis are less than one hundred miles apart, there is quite a difference in the average temperatures of the two places. At Davis the temperature frequently reaches  $100^{\circ}$  F, while at Palo Alto it is usually not more than  $80^{\circ}$  to  $85^{\circ}$  F. The data on metaphase pairing in these two plants are given in table 3; number 1 was at Palo Alto, and number 2 at Davis. There is very little difference in the percentage of each type of pairing observed. This fact suggests that the occurrence of meiotic irregularities in these similar  $F_2$  plants was not affected by such differences in temperature as were involved. It seems probable, therefore, that this temperature range ( $80^{\circ}$ – $100^{\circ}$  F) may be included within a certain optimum range for meiotic regularity in these hybrids.

Later, the twelve achenes from  $F_2$  plant number 2 were planted, and eleven seedlings were produced. At the same time, larger numbers of other *Crepis* seeds were planted, and all populations were transplanted into 4-inch pots in the same type of soil. The eleven seedlings from plant number 2 grew very rapidly and soon produced fine rosettes. They did not produce many roots, however, and finally the entire population became chlorotic and died. An examination of the plants a few days before death showed a very meager root system as compared to top growth. The other *Crepis* plants on the same bench grew vigorously. Only a few root tips were obtained from three of these  $F_2$  plants and they were in poor condition. They showed that these three plants had 8 chromosomes; but the figures were so poor that identification was impossible. Definite conclusions concerning viability of the polyploid pollen cannot be drawn from such meager data, but Matsuda (1927) has shown that large pollen grains of *Petunia* are much less viable and slower to germinate than are normal ones.

#### INTRASPECIFIC RELATIONS AND MEIOTIC BEHAVIOR

The differences in amount of pairing at metaphase in hybrid A and hybrid B (table 3) show that the chromosomes of *C. setosa Topaliana* fail to form bivalents at metaphase with the *nicaensis* chromosomes as

regularly as do the chromosomes of *C. setosa typica*. Also, when bivalents occur, the association of the paired chromosomes is generally less intimate in hybrid B than in hybrid A. That is, there are more bivalents in hybrid B showing merely terminal association. Such differences in meiotic behavior, however, do not necessarily indicate wide genic diversity between these two forms of *C. setosa*, because comparable differences have been found among closely similar individuals of a single species and in hybrids involving less diverse forms of a species.

Plants of a certain race of *C. capillaris* known as the X strain are morphologically typical of the species and closely similar to one another except for minor variations. Hollingshead (1930) found univalents at metaphase in from 12 to 44 per cent of PMC's in different plants of this strain growing under the same favorable conditions. More recently it has been discovered (Richardson, 1935) that, in progeny from a diploid branch of a haploid individual of this same strain of *C. capillaris*, the occurrence of univalents at IM is caused by failure of chiasma formation between homologous chromosomes *which are found to be regularly associated at pachytene*. She reports that such univalent homologous chromosomes frequently lie in juxtaposition at diakinesis and this is thought to be a result of their earlier association. Whatever the cause of failure in chiasma formation may be, the fact remains that marked irregularities in metaphase pairing may occur in similar individuals of the same species. It has been found by Beadle (1930) and others in several species of plants that such differences between individuals may be caused by a single gene difference.

Furthermore, Hollingshead (1930) studied the occurrence of irregularities in metaphase pairing in hybrids between *C. capillaris* ( $n=3$ ) and *C. tectorum* ( $n=4$ ). In these experiments the X strain of *C. capillaris* was crossed with two strains of *C. tectorum* (1498 and 1648). These two strains of *tectorum* were similar in most respects, but it was noted by Hollingshead that the 1498 strain was more uniform and had descended from one plant, whereas the 1648 strain was grown from wild seed and varied in leaf shape and time of maturity; also that 1498 came from the Copenhagen Botanic Garden, but that 1648 was collected near Tomsk, Siberia. Although the original source of the Copenhagen strain is not known, it is probable that the two strains represent widely separated geographic races. Furthermore, the achenes of these two strains are significantly different in width and possibly in length. At any rate, the Siberian strain has relatively narrow achenes. Such a difference is sufficient to warrant the recognition of these strains as distinct forms or perhaps as varieties, but they are certainly not sufficiently distinct in morphology to admit of their being recognized as subspecies. Yet the difference in meiotic irregularities in the hybrids between these two

strains of *tectorum* and the same strain of *capillaris* are even greater than the corresponding differences between the hybrids involving the two subspecies of *setosa*. This is shown in table 5, in which the original data are made more easily comparable by stating the percentages of PMC's showing the various numbers of pairs at metaphase. From these data it appears that hybrids 1A and 1B show less regular pairing at metaphase than do hybrids 2A and 2B. But this may be entirely due to the known tendency to meiotic irregularity in the *capillaris* X strain and not to a difference in the two *tectorum* strains. This may also account for the apparent tendency to greater difference in amount of meiotic

TABLE 5

COMPARISON OF MEIOTIC IRREGULARITIES IN CAPILLARIS-TECTORUM HYBRIDS AND IN NICAENSIS-SETOSA HYBRIDS

| Hybrids   | Per cent of PMC's with |     |     |     |     |
|---|------------------------|-----|-----|-----|-----|
|   | 4ii                    | 3ii | 2ii | 1ii | 0ii |
| 1A. <i>capillaris</i> × <i>tectorum</i> 1498.....   | ..                     | 55  | 36  | 8   | 1   |
| 1B. <i>capillaris</i> × <i>tectorum</i> 1648. ....  | ..                     | 18  | 30  | 25  | 27  |
| 2A. <i>nicaensis</i> × <i>setosa</i> Topaliana..... | 91                     | 7   | 1   | 1   | 0   |
| 2B. <i>nicaensis</i> × <i>setosa</i> typica.....    | 57                     | 27  | 14  | 2   | 0   |

irregularity between 1A and 1B than between 2A and 2B. From these data, therefore, no conclusion can be drawn concerning the bearing of the amount of pairing at metaphase on the degree of genetic relationship between two subspecific entities.

Other differences were found, however, between the two subspecies of *C. setosa*, besides the difference in behavior of their chromosomes when associated with *nicaensis* chromosomes in hybrids. The morphological differences already noted (table 1) justify their rank as subspecies, and the behavior of their chromosomes in the hybrids discussed above is consistent with this classification. Babcock and J. Clausen (1929) have shown that the closer the phylogenetic relationship between the species used in a cross, the higher the percentage of good pollen grains and the greater the fertility of the hybrid. The pollen counts of the hybrids, given in table 3, show a very striking difference. The percentage of meiotic irregularities observed in hybrid A, as compared with those in hybrid B, is of nearly the same ratio as the numbers of seed produced by each hybrid. Although their chromosomes correspond closely in size and shape, these subspecies of *setosa* may have had their chromosomal or-



ganization greatly altered. The fact that attempts to cross them were unsuccessful might indicate such a situation, but such negative results are of slight value in judging of relationships. The use of other forms of the same subspecies might possibly result in hybrids. If they do seldom cross in the regions where their ranges overlap, this would be an important factor in the divergence of the two entities. The point of importance to taxonomy is the fact that two entities exist which appear to have diverged from one species and which differ in several morphological details, but which are still so similar that practical considerations oppose their being recognized as distinct species. When such a situation is found, together with intergrading forms in the region of overlapping, the most satisfactory systematic disposition of these forms is their treatment as subspecies.

## A REVIEW OF THE EVIDENCE ON MEIOSIS IN INTERSPECIFIC HYBRIDS OF CREPIS WITH REFERENCE TO INTRAGENERIC RELATIONS

The three major subdivisions of the genus, namely, *Catonia*, *Eucrepis*, and *Barkhausia*, may be considered as natural in the sense that, as a general rule, the species within each subgenus are more closely related to each other than they are to species in the other two subgenera. But, as has already been pointed out (Babcock, 1936), the very close relationship between the subgenera, as indicated by comparative morphology, makes it difficult to separate them by hard and fast lines. Thus certain species of *Eucrepis* resemble *Catonia* in their relatively unspecialized involucre, while several others approach *Barkhausia* in the structure of their fruits. Also, at least one species of *Eucrepis* shows certain affinity with species in both the other subgenera. This particular species, *C. leontodontoides*, was involved in the five interspecific hybrids studied by Avery (1930). Its relationships to the other species involved in these hybrids are considered below. The point to be emphasized here is that *C. leontodontoides* is a bridging species between *Catonia*, the most primitive, and *Barkhausia*, the most advanced, of the three subgenera. Concerning the genus as a whole, the evidence from chromosome number and morphology (Babcock and Cameron, 1934) and from geographic distribution (Babcock, 1936) also indicates the close interrelationship between the three subgenera and suggests a monophyletic origin for the genus, or at least that it is a natural group which arose from a common progenial stock.

The study of interspecific hybrids in *Crepis* has provided additional evidence bearing on phyletic relations within the genus. In the early work of Collins and Mann (1923) the first occurrence of autosyndesis was discovered in the hybrid *C. setosa*  $\times$  *C. biennis*. As the 20 chromosomes of *biennis* formed 10 pairs at IM the 4 *setosa* chromosomes were distributed at random. Thus metaphase pairing furnished only negative evidence on the relationship between these species. Subsequent work on this hybrid led to the discovery that *C. biennis* is an octoploid species with a base number of 5, which was later confirmed by direct study of the somatic chromosomes (Babcock and Swezy, 1935). In another hybrid, *C. capillaris*  $\times$  *C. setosa* (Collins and Mann, 1923), it was first reported that no pairing of the chromosomes was observed and that the fertility was extremely low. But it was reported later by Hollingshead (1930; based on unpublished data of Mann Lesley on a hybrid involving different strains of *capillaris* and *setosa*) that variable pairing was observed. It is possible that a small amount of metaphase pairing occurred

in the original hybrid of Collins and Mann. Yet another hybrid, *C. capillaris*  $\times$  *C. aspera*, has been reported (Navashin, 1927) as very irregular in meiosis, with random distribution of unsplit or split univalents; but possibly a small amount of pairing also occurs in this hybrid. The occurrence of pairing in all other interspecific *Crepis* hybrids examined certainly warrants the recognition of this possibility.

Most of the other studies of meiosis in *Crepis* hybrids were made before the relations between pachytene chiasmata and pairing at metaphase had been worked out (Darlington, 1932) and the few attempts to study the premeiotic prophases in *Crepis* hybrids had proved unsuccessful. The recent contributions of Richardson (1935a, b) on meiosis in *Crepis* have shown that the striking individuality of the chromosomes and their meiotic behavior in species with low numbers offsets the technical difficulties involved in the small size of the florets and the paucity of pollen mother cells. Koller (1935) investigated meiosis in *Crepis aurea* ( $n=5$ ) of subgenus *Catonia* and *C. rubra* ( $n=5$ ) of subgenus *Barkhausia* and found that there is a definite difference with respect to the initial chiasma frequency and the degree of terminalization. It was concluded that the chromosome complements of two taxonomically well-differentiated species of the same genus may be similar in external structure, but dissimilar in behavior in meiosis, thus showing the important internal differences between these species.

Hitherto, the study of meiosis in species hybrids in *Crepis* has been restricted to the counting of bivalents and univalents appearing at metaphase. In these studies the proportion of bivalents observed in the various hybrids has been regarded as an indication of the degree of genetic affinity between the parent species. According to Darlington (*op. cit.*, pp. 159-160), amount of pairing between hybrids of various species cannot be compared as an indication of genetic similarity without a knowledge of chiasma frequencies in the parents; and differences in amount of pairing will have a very indirect relation to the differentiation in genetic properties of the parent species. Accurate data on chiasma frequency and behavior in parents and hybrids are no doubt essential for the elucidation of metaphase association in hybrids characterized by a mediocre or low amount of pairing. But if persistent chiasmata are essential for bivalent association at metaphase, then the amount of metaphase pairing must directly result from the number of such chiasmata. Also the abundance of evidence that pairing of chromosomes at pachytene is usually determined by the conjugation of homologous chromosomes justifies consideration of the occurrence of a number of bivalents at metaphase as direct evidence of genetic homology between the parent species. Moreover, when high and low amounts of pairing at metaphase are positively correlated with other evidence on genetic relationship,

these differences in pairing may be considered as consistent and cogent evidence on relationship of the parent species. With these considerations in mind, it seems worth while to reexamine the data on chromosome pairing in all the interspecific hybrids of *Crepis* in which such studies have been made.

In order to compare the evidence thus far available from the study of these hybrids with the other categories of evidence already mentioned, comparable data on the meiotic behavior of the chromosomes in  $F_1$  plants from eleven different interspecific crosses have been assembled in table 6. In connection therewith, references are given to the original papers. In this table the proportions of PMC's with various numbers of bivalents are expressed as percentages of the whole number of PMC's in which it was possible to make counts and, in order further to facilitate comparison of the hybrids, the mean numbers of bivalents per PMC are given. The classification of the species in subgenera is indicated typographically as follows: *Catonia*, capitals; *Eucrepis*, bold face; *Barkhausia*, italics. The phylogenetic relations of the species are roughly indicated by four categories in ascending order, viz.: primitive, less primitive, less advanced, advanced. It is unnecessary to go into detail here concerning the basis of classification into these categories. The features most used are life cycle, size of plant, shape of leaves, size of heads, size of outer involucre bracts, modification of inner bracts, size of florets, size and shape of fruits, number and size of chromosomes. It is believed that the classification here given is a fair representation of the relative phylogenetic position of the several species. Phyletic relations as based on morphology alone, however, are only approximate and the classification of the species into the four categories is more or less arbitrary.

Three of the hybrids, numbers 1, 9, and 11, are preëminent in the large amount of pairing exhibited. In each hybrid one of the parent species is more or less primitive and the other parent is in the next higher class. These facts are consistent with the idea that in more primitive species there have been relatively fewer genetic changes than in more advanced species. But hybrid 10 is like hybrids 9 and 11 with respect to phyletic classification of the parent species, and hybrid 10 shows a comparatively low amount of pairing. A brief consideration of the specific relations between the parents of each hybrid will throw light on this situation.

In hybrid 1, although *aurea* is of *Catonia* and *leontodontoides* of *Eucrepis*, both species are perennials, and they are rather similar in certain morphological features, particularly in size and shape of the fruits. Also they both have 5 pairs of chromosomes which are closely similar in size and shape.<sup>1</sup> Furthermore, they are indigenous in the same

<sup>1</sup>All comparisons of karyotypes are based on Babcock and Cameron (1934).

TABLE 6  
COMPARISON OF CHROMOSOME PAIRING AT METAPHASE IN ELEVEN INTERSPECIFIC *Crepis* HYBRIDS WITH REFERENCE TO  
SUBGENERIC CLASSIFICATION AND RELATIVE PHYLETIC STATUS OF THE SPECIES INVOLVED

(Classification of species into subgenera indicated by capitals (*Catonia*), boldface (*Eucrepis*), or italics (*Bartisauria*). Haploid chromosome numbers stated.)

| No.* | Primitive | Less primitive      | Less advanced              | Advanced               | Per cent of PMC's with |          |          |          |         |         | Total PMC's | Mean number of bivalents per PMC |
|------|-----------|---------------------|----------------------------|------------------------|------------------------|----------|----------|----------|---------|---------|-------------|----------------------------------|
|      |           |                     |                            |                        | 5n                     | 4n       | 3n       | 2n       | 1n      | 0n      |             |                                  |
| 1    | AUREA 5   | leontodontoides 5   |                            |                        | 74                     | 23       | 3        | 0        | 0       | 0       | 53          | 4.7±.16†                         |
| 2    |           | leontodontoides 5   | tectorum 4                 |                        | ..                     | 20       | 15       | 26       | 19      | 20      | 54          | 2.0±.26                          |
| 3    |           | leontodontoides 5   | parviflora 4               |                        | ..                     | 42       | 15       | 20       | .04     | 22      | 27          | 2.5±.47                          |
| 4    |           | leontodontoides 5   | capillaris 3               |                        | ..                     | ..       | 20       | 20       | 16      | 44      | 25          | 1.5±.25                          |
| 5    |           | leontodontoides 5   |                            | <i>Marchalii</i> 4     | ..                     | 9        | 22       | 23       | 16      | 30      | 56          | 1.6±.22                          |
| 6    |           |                     | capillaris 3<br>tectorum 4 |                        | ..                     | A<br>B   | 55<br>18 | 36<br>30 | 8<br>25 | 1<br>27 | 442<br>576  | 2.5±.09<br>1.4±.05               |
| 7    |           |                     | tectorum 4                 | <i>taraxacifolia</i> 4 | ..                     | 30       | 27       | 37       | 6       | 0       | 30          | 2.8±.27                          |
| 8    |           |                     | <i>aspera</i> 4            | <i>bursifolia</i> 4    | ..                     | 19       | 40       | 19       | 14      | 8       | 59          | 2.5±.28                          |
| 9    |           | <i>aculeata</i> 4   | <i>aspera</i> 4            |                        | ..                     | 61       | 31       | 8        | 0       | 0       | 36          | 3.5±.18                          |
| 10   |           | <i>divaricata</i> 4 | <b>Dioscoridis</b> 4       |                        | ..                     | 10       | 14       | 30       | 37      | 9       | 57          | 1.8±.23                          |
| 11   |           | <b>nicaeensis</b> 4 | <i>setosa</i> 4            |                        | A<br>B                 | 91<br>57 | 7<br>27  | 1<br>14  | 1<br>2  | 0<br>0  | 124<br>114  | 3.9±.21<br>3.4±.18               |

\* Basic data for numbers 1 to 5 from Avery (1930) and for number 3 additional unpublished data; for number 6 from Hollingshead (1930); for numbers 7 to 9 from Babcock and Clausen (1929); and for number 10 from Muntzing (1934).

† Standard error.

geographic area, although *aurea* is a montane species and *leontodontoides* occurs at low altitudes. This evidence, together with the fact of high degree of pairing in the hybrid, warrants the inference that although *leontodontoides* has developed the more specialized involucre of *Eucrepis* and in other respects is a less primitive species than *aurea*, yet there is a high degree of homology between the chromosomes of the two species.

The parents of hybrid 9, *aculeata* and *aspera*, are in the same section of *Barkhausia* and are obviously closely related; but *aculeata* is somewhat more primitive, having larger heads, florets, and fruits, less differentiated marginal achenes, and similar but much larger chromosomes. Therefore a high degree of genic homology may be assumed, and an even larger amount of pairing at metaphase than was observed might be expected. As 61 per cent of the PMC's had  $4_{II}$  and 31 per cent  $3_{II}$ , it is possible that the difference in size or some other structural difference prevented chiasma formation or terminalization in one particular chromosome type. A study of pachytene and later stages in this hybrid would be of interest. The two species occur in the same geographic area.

Hybrid 11 is the subject of the first part of the present paper, and the differences between 11A and 11B in number of pairs at metaphase have been discussed. At this point only hybrid A will be considered. Although *nicaeensis* is of *Eucrepis* and *setosa* of *Barkhausia*, and although *nicaeensis* is somewhat more primitive, being biennial, larger throughout, and with thicker, beakless fruits, nevertheless the two species are generally similar. This is true especially when *setosa typica* is compared with *nicaeensis*, and when habit, type of involucre, and size of heads, florets, and fruits are considered. The chromosomes are of the same four types, and in only one of the types do the representatives of the two species differ greatly in size. Furthermore, *setosa typica* is indigenous in low altitudes of the same geographic area as *nicaeensis*, which is montane. Hence the nearly complete metaphase pairing and the closeness of the association in the bivalents can be interpreted as indicating a high degree of genic homology in these species.

In hybrid 10 a quite different situation exists. The phyletic relation is the reverse of that in number 11, the *Barkhausia* species, *divaricata*, being somewhat more primitive than the *Eucrepis* species, *Dioscoridis*. Both species have 4 pairs of chromosomes. But on the basis of comparative morphology they are widely separated; for they differ throughout and notably in that *divaricata* has less reduced outer involucre bracts and monomorphic, shortly beaked achenes; whereas *Dioscoridis* has a rather highly specialized involucre and dimorphic, unbeaked achenes. The two species are widely separated geographically, *divaricata* being endemic in Madeira, and *Dioscoridis* in Greece. The amount of chromo-

some pairing in this hybrid is practically the same as in hybrid 5. In the latter the parents were *leontodontoides*, a relatively primitive species of *Eucrepis* with 5 pairs of chromosomes, and *Marschallii*, an advanced species of *Barkhausia* with 4 pairs. These species are indigenous in widely separated regions, *leontodontoides* in Italy and southwestern France, *Marschallii* in the Caucasus and Caspian region. All this evidence indicates that in both of these hybrids the parents represent widely divergent phyletic lines within the genus and is consistent with the notion that the low amount of metaphase pairing in these two hybrids indicates relatively low amounts of genic homology.

Hybrids 2, 3, and 4 may be considered together since the phyletic relations are similar. Unfortunately the data on numbers 3 and 4 are very limited and in number 3 the distribution of PMC's according to number of bivalents present is irregular. In all three hybrids both parents are *Eucrepis* species, and the more primitive one is *leontodontoides*, which is indigenous in Italy and southwestern France. The natural distribution of the other three species is as follows: *tectorum*, middle and northern Eurasia; *parviflora*, eastern Mediterranean to Caucasus; *capillaris*, southern and middle Europe east to Crimea. The mean numbers of bivalents per PMC are: hybrid 2, 2.0; hybrid 3, 2.5; hybrid 4, 1.5. The amount of pairing in number 2 is consistent with the view that, in *Crepis*, species which are widely separated in geographic distribution have less genetic homology than species occupying the same region. The slightly larger amount of pairing in hybrid 3, of which the parents are more widely separated geographically, is in apparent disagreement with this conception, but the small number of PMC's counted and their irregular distribution according to number of bivalents present, make this apparent exception less significant. Hybrid 4 also appears to be an exception to the hypothesis because the geographic areas of the parents are the closest in this hybrid, yet the amount of pairing is the lowest. But such an inference is not warranted for this hybrid, because the *capillaris* plants used as parents were all of the X strain in which wide variations in pairing occur between individuals.

Hybrids 6, 7, and 8 may also be considered together. Although in number 6 two forms of *tectorum* were used, the differences in pairing observed were not comparable to those obtained in hybrid 11 because of the known variability of pairing in the *capillaris* plants used in this cross. For the same reason hybrid 6 cannot be compared with hybrids 7 and 8 with any degree of assurance. It may be noted, however, that the amount of pairing in number 6A is more nearly what might be expected from the morphological similarity of the two species and their geographic distribution. Hybrids 7 and 8 can be compared with more assurance. All four parental species have 4 pairs of chromosomes. Although number 7

was a hybrid between a *Eucrepis* species and a *Barkhausia* species and number 8 involved two *Barkhausia* species, the phyletic relations between the two pairs of species are similar in that one species is less advanced than the other. The two hybrids differ, however, in the degree of difference in advancement of the parents. In other words, the degree of advancement of *taraxacifolia* over *tectorum* is greater than that of *bursifolia* over *aspera*. But the amount of pairing in the two hybrids is equal, the slight difference between them not being significant. This apparent inconsistency is offset by the evidence from geographic distribution. In number 7 the areas occupied by the two species overlap, but in number 8 one parent (*aspera*) occurs in Asia Minor and the other (*bursifolia*) is endemic in Italy. Therefore the geographic distribution of the two pairs of species seems to correspond with their degree of genic homology, as indicated by metaphase pairing in their respective hybrids, if allowance be made for the difference in phyletic relations.

In the foregoing review the degree of genetic homology between pairs of species, as indicated by the number of bivalents at IM in their respective hybrids, has been considered with reference to the relative phyletic status of the species and their geographic distribution. The hybrids investigated are too few to warrant any conclusions, and the crossings were not planned with reference to a study of the relation of metaphase pairing to either phyletic status or geographic distribution considered alone. But, if both these variables are taken into account when the metaphase pairing in two hybrids is compared, two inferences appear to be indicated, as follows: (1) hybrids between more primitive species have larger amounts of metaphase pairing than hybrids between more advanced species; (2) hybrids between species occupying the same geographic region also exhibit greater meiotic regularity than do hybrids between species from widely separated regions. It is to be emphasized that these inferences apply only to the *Crepis* hybrids herein considered.

That hybrids between more primitive species of a monophyletic genus will exhibit greater meiotic regularity than do hybrids between more advanced species, is a reasonable *a priori* assumption, because the more primitive species would be less differentiated genetically in a truly natural group. From a study of meiotic behavior in *Nicotiana* interspecific hybrids, Goodspeed (1934) reached the conclusion that formation of bivalents in a hybrid is positive evidence that the pairing chromosomes possess many similar or equivalent genic elements; and that decrease in amount of pairing "is to be related to an accumulation of genic and chromosomal distinctions between species evolved from a common stock." The data on *Crepis* hybrids are not adequate for any generalization about phyletic status and meiotic regularity. There should be several series like hybrids 1-5, all of which involve a single species for one



parent, and both parents should occur in the same region. Even in this series numbers 3 and 4 must be disregarded because of unreliability of the data, and in number 5 the factor of wide geographic separation is introduced. This leaves only the first two hybrids, in which there is a significant difference in amount of pairing and a great difference in phyletic status of *aurea* and *tectorum*. The more primitive *aurea* produced a hybrid with high meiotic regularity and high fertility, and the more advanced *tectorum* gave a hybrid with low metaphase pairing and extremely low fertility.

With reference to the second inference, it seems probable that, in a recently evolved genus with many closely related but widely separated species, there is positive correlation between wide geographic distribution and a low degree of genetic homology. This also is a reasonable assumption because groups of most closely related species would be distributed from the center or origin during the course of evolution. In the present study hybrids 8 and 9 support this inference because all four species are of subgenus *Barkhausia*, the parents of hybrid 8 being widely separated geographically and less similar morphologically, whereas the parents of hybrid 9 occur in the same region and are generally similar. Hybrid 8 has a mean of  $2.5 \pm .28$  bivalents per PMC, and hybrid 9 has a mean of  $3.5 \pm .18$ . The difference,  $1.0 \pm .33$ , is three times its standard error and hence may be considered significant. A similar situation holds in hybrids 10 and 11 and in these there is a much greater difference in the number of bivalents formed in the hybrids. It should be emphasized, however, that this positive correlation between wide geographic separation and relatively low genetic homology is suggested only for the genus *Crepis* and, perhaps, for other large, recently evolved, but widely distributed genera.

As a matter of fact, exactly the opposite relation has been reported in certain genera, such as *Philadelphus* (Bangham, 1929; Sax, 1931) and *Platanus* (Sax, 1933). Both of these are old genera. According to Seward (1931), *Platanus* is one of the oldest of the broad-leaved trees, and we are informed by Chaney (*in litt.*) that there is no question of its presence in early Tertiary rocks in many parts of the world, including western North America. With respect to *Philadelphus*, Chaney (1927) has recognized it in middle Tertiary deposits of eastern Oregon, and Cockerell (1908) has recognized it in the Upper Miocene of Colorado. At all events *Platanus* and *Philadelphus* are certainly more primitive and certainly much older than *Crepis*. In both those genera hybrids have been obtained between certain Old World and New World species having the same chromosome number, and some of these hybrids exhibit complete or nearly complete pairing of the chromosomes, although there is much variation among them with respect to fertility. The perfect or

nearly perfect pairing at diakinesis or IM in these hybrids indicates a high degree of genetic homology in the parental species. Apparently the chromosomes of these widely separated species have undergone insufficient change to prevent pairing although the species have been isolated for millions of years. In the present discussion it is unnecessary to consider the problem of stability and plasticity in species and genera. It is sufficient to note that such differences exist and that relations between geographic distribution and genetic homology of species will vary accordingly.

Summarizing the evidence on intrageneric relations in *Crepis* leads to certain generalizations about this genus. *Crepis* is a large group of closely related species. Although natural major subgeneric groups are clearly indicated and these groups provide a convenient basis for systematic classification, yet the species thus classified under different subgenera are still more or less closely related. That is, their genic complements are more or less homologous. This is certainly indicated by the evidence on metaphase pairing in the interspecific hybrids reviewed above. It is supported also by two other categories of evidence. (1) It has already been shown (Babcock and Cameron, 1934; Babcock, 1936) that in several groups of morphologically similar species there is a single karyotype even though the species occupy different geographic areas. Evidently differentiation and distribution has not been accompanied in these groups by visible marked changes in the chromosomes. Thus the inference seems warranted that speciation has been made possible by a limited number of gene mutations or minute structural changes, leaving the main part of the residual genotype unchanged. (2) This inference is shown to be valid by the results of the investigations on three such groups of species made by Jenkins, Cave, and Smith independently (unpublished data). In each group the interspecific hybrids studied exhibited almost complete pairing at metaphase; and genetic experiments with alternative characters in different species proved that these characters are inherited in Mendelian fashion. In general, therefore, the evidence on metaphase pairing in interspecific hybrids in *Crepis* is consistent with all the other evidence on phylogeny in this genus.

## SUMMARY AND CONCLUSIONS

1. Two subspecies of *Crepis setosa*, namely, *typica* from western Europe and *Topaliana* from Greece, were used in this study. They had been ranked as subspecies because of the differences they exhibited in external morphology. Each subspecies was used as the paternal parent in a cross made with the same plant of *C. nicaeensis*. The diploid chromosome number in both species is 8. The morphology of the chromosomes of the *setosa* subspecies is apparently the same, and they differ sufficiently from the members of the *nicaeensis* genom to make identification in  $F_1$  relatively easy.

2. The interspecific hybrids were produced with difficulty, and only one plant from each cross was available for cytological study. These are designated as hybrid A (*nicaeensis* ♀ × *setosa typica* ♂) and hybrid B (*nicaeensis* ♀ × *setosa Topaliana* ♂).

3. The satellite of the *nicaeensis* D chromosome was not found in either  $F_1$  plant, and in each plant there was an increase in the size of the head of this chromosome.

4. Meiosis in the  $F_1$  plants showed that the chromosomes of *setosa typica* paired very regularly with the *nicaeensis* chromosomes and exhibited a very close association of chromosomes in bivalent formation, whereas those of *setosa Topaliana* exhibited less regular pairing and looser association. Percentage of good pollen grains was determined and showed a high correlation with meiotic behavior, being much greater in hybrid A than in hybrid B. Comparative fertility under open pollination was consistent with the foregoing evidence.

5. An  $F_2$  population of 87 plants was grown from hybrid A, and one of 17 plants from hybrid B. In the first group, eighty-four plants were found to contain 24 chromosomes and three plants had 8 chromosomes; in the second group, fifteen plants had 24 chromosomes and the remaining two plants had 8 chromosomes. The 24-chromosome plants were found to have arisen by spontaneous crossing with *Crepis biennis*. With the exception of but six plants, they were all biennial. Each of the six that bloomed the first year was slightly fertile under isolated conditions.

6. The  $F_2$  plants with 8 chromosomes were studied carefully because of their unusual chromosome complement. The morphology of these 8 chromosomes indicated that 6 were from *setosa*—one A, two B, one C, and two D; and the two remaining were apparently *nicaeensis* D chromosomes. This supposition, however, was not supported by meiotic behavior, either in  $F_1$  or in  $F_2$ ; and it was suggested that an exchange of material occurred in  $F_1$  and that the  $F_2$  chromosomes represent recombination products of this exchange.

7. The various stages in the formation of giant pollen grains in  $F_2$  plants with 8 chromosomes were observed. But polyploids were not obtained in  $F_2$ , primarily because of high sterility of the  $F_2$  plants and low viability of the  $F_2$  progeny. Slower germination of the polyploid pollen may have been a secondary factor.

8. The two subspecies of *Crepis setosa* are so similar morphologically that their classification as different species does not seem to be justified. Although their chromosomes are exactly similar in appearance, yet they must differ somewhat in genetic composition. This is indicated both by the morphological differences between the subspecies, and by the differences in metaphase pairing in hybrids A and B. Classification as subspecies, at least for the present, has the advantage of emphasizing their very close affinity.

9. The genus *Crepis* is a group of closely related species. The subgenera, *Catonia*, *Eucrepis*, and *Barkhausia*, are not widely differentiated, but overlap more or less, and several hybrids have been produced between species of different subgenera. Eleven interspecific hybrids between diploid species have been studied by various investigators with particular reference to the amount of pairing at metaphase. The evidence indicates that the genic complements of the fourteen species involved are all more or less homologous. This inference is consistent with the evidence on chromosome morphology in the genus, on geographic distribution, and on other results (as yet unpublished) from genetic investigations on groups of closely related species. The evidence on metaphase pairing in interspecific hybrids, therefore, supports the conception that the subgenera of *Crepis* had a common origin and are still more or less similar in genetic composition.

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## EXPLANATION OF PLATES



## PLATE 14

### Fruiting heads

*a. C. scitosa typica* (2623).

*b. C. scitosa Topaliana* (2671).

*c. C. nicaricensis* (2700).

*d. C. nicaricensis* ♀ × *C. scitosa typica* ♂ F<sub>1</sub> (30.31).

*e. C. nicaricensis* ♀ × *C. scitosa Topaliana* ♂ F<sub>1</sub> (30.32).

### Somatic chromosomes

*f.* Photomicrograph of mitotic metaphase in a root-tip cell of an 8-chromosome F<sub>2</sub> plant.

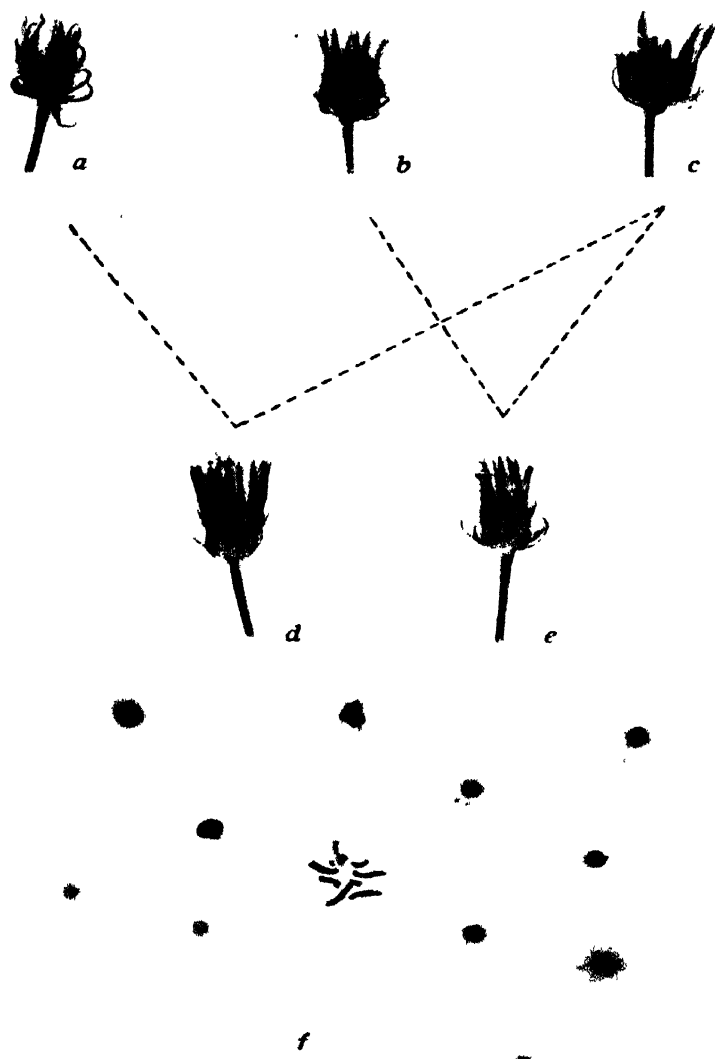


PLATE 15

(*Crepis nicaeensis*  $\times$  *C. setosa typica*)  $\varphi \times C. biennis$   $\sigma$

*a-e.* Basal leaves from five 24-chromosome plants derived from open-pollinated  $F_1$ .

*Crepis nicaeensis*  $\times$  *C. setosa typica*,  $F_2$  progeny.

*f, g.* Two of the 8-chromosome plants.

*h, i.* Two of the plants that died in early rosette stage, before the chromosomes were examined.



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## INTRODUCTION

The cytogenetical investigations designed to throw more light on relationships and phylogeny of the various species in *Crepis* have progressed along two lines: first, an examination of the chromosomes of the various species; and second, a study of hybrids. For the most part, the study of hybrids has been confined to those between more distantly related species, which, in the main, have been sterile. Consequently, the emphasis has been upon the cytology of the  $F_1$  hybrids rather than upon the genetical basis of the differences between the parental species.

There are, however, a number of species groups the members of which are closely related morphologically and have a similar karyotype (Babcock and Cameron, 1934). From the morphological evidence, these species have had a common origin and apparently have not diverged very far from one another. The obvious conclusion is that the similar chromosome morphology indicates, in such closely related groups in *Crepis*, a fundamental similarity of the genes and their arrangement in the various chromosome types.

The present paper deals with such a closely related group of species in *Barkhausia*, the most advanced subgenus of *Crepis*. Three of these species are insular endemics of Madeira and the Canary Islands; the fourth is a widespread species of northern Africa and Europe, which includes one endemic and one introduced subspecies in Madeira.

The three endemic species are *Crepis divaricata* Lowe, *C. Noronhaea* Babc.,<sup>1</sup> and *C. canariensis* (Sch. Bip.) Babc.<sup>2</sup> The fourth species is *C. vesicaria* L., and the two subspecies dealt with in this investigation are *C. vesicaria taraxacifolia* (Thuill.) Thell. and *C. vesicaria andryaloides* (Lowe) Babc.<sup>3</sup>

<sup>1</sup> *Crepis Noronhaea* nom. nov. = *Borkhausia* (sic) *divaricata* var. *pumila* Lowe, Trans. Camb. Phil. Soc., 4:26, 1831; non *C. pumila* Rydb., Mem. N. Y. Bot. Gard., 1:426, 1900. Named for Sr. A. C. de Noronha, Director, Museu Regional, Funchal, Madeira, who sent the seed, collected in Porto Santo, from which experimental cultures were grown.

<sup>2</sup> *Crepis canariensis* (Sch. Bip.) comb. nov. = *C. Lowei* var. *canariensis* Sch. Bip. ex Webb et Berth., Phyt. Canar., 3:461, 1836-1850; *Barkhausia hieracioides* Lowe ex Webb et Berth., l.c. det. apud Lowe in litt., sed cf. Lowe, Fl. Mad., 1:559, 1868.

<sup>3</sup> *Crepis vesicaria* subsp. *andryaloides* (Lowe) comb. nov. = *C. andryaloides* Lowe, Trans. Camb. Phil. Soc., 4:25, 1831; *Borkhausia* (sic) *hieracioides* Lowe, op. cit., p:27, no. 44; *B. dubia* Lowe, l.c., no. 45; *B. comata* Lowe, l.c., no. 46; *C. comata* Banks et Sol. ex Lowe, l.c.; *Barkhausia hieracioides* et *dubia* (Lowe) DC., Prod., 7:157, 1838; *C. hieracioides* et *dubia* F. Schultz, Flora, 23:718, 1840; *C. auriculata* Sol. ex Lowe, Man. Fl. Mad., 1:556, 1868.



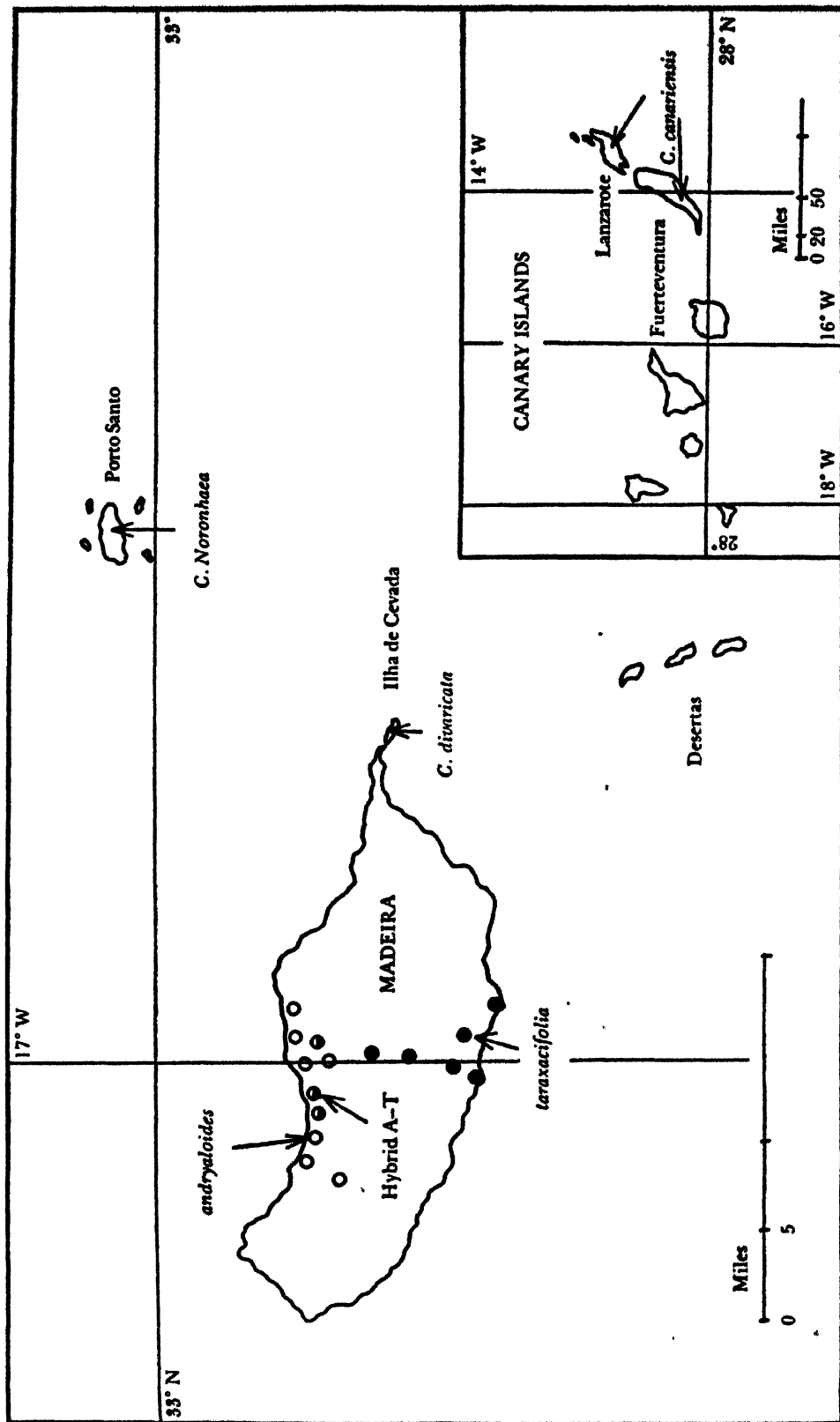


Fig. 1. Map of Madeira and the Canary Islands showing the distribution of the species and subspecies.

*Crepis divaricata* is found only on the eastern promontory of Madeira and there it is nearly extinct, owing to overgrazing by goats. *C. Noronhaea* is known only from Porto Santo Island, which lies to the east of Madeira. Its chromosomes were reported on by Babcock and Cameron (1934) under the name *C. pumila*, but this name is invalid. *C. canariensis* occurs on the two easternmost of the Canary Islands, Lanzarote, where it is abundant, and Fuerteventura.

*Crepis vesicaria andryaloides* is also endemic in Madeira, being found only in the mountains along the north coast and occasionally on the steep slopes exposed to the sea down which it is carried by wind or water. It was finally recognized by Lowe (cf. Man. Fl. Mad. under *C. hieracioides* and *C. andryaloides*) as a highly variable species with many intergrading forms, some of which were so extreme that he had previously given them specific or varietal names. For sake of brevity it will be referred to in this paper as *andryaloides*.

*Crepis vesicaria taraxacifolia* is distributed in northwestern Africa and western Europe. It is polymorphic and several of its forms have been given specific names. A form which occurs in Portugal was described as a species (*C. intybacea*) by Brotero in 1816 and this form seems to have been introduced by the Portuguese into Madeira at an early date. There it was found and described by Lowe in his Manual (1868) as *C. laciniata* with two varieties, *pinnatifida* and *integrifolia* (the latter occurring here and there with the former, but less commonly). *Taraxacifolia* is abundant around Funchal, the only port on the island, and in the vineyards around Boa Ventura on the north coast. Since it was found by Babcock along the trail above Boa Ventura, it is inferred that it has spread from Funchal to the north coast by this route. But it was not seen at all in the central highlands, so it probably has been carried by man or animals. The important point is that, having arrived on the north coast, it is hybridizing freely with *andryaloides* where the two come in contact; and it now seems probable that some of Lowe's perplexing forms (*dubia*, *comata*, and even the type of *andryaloides*) were the products of earlier hybridization.

The main object of the present investigation was to study the five species or subspecies from as many different points of view as possible and particularly to state their relationships in cytogenetic terms.

#### ACKNOWLEDGMENTS

This study was begun in 1931 at the suggestion of Professor E. B. Babcock, who supplied the material and facilities for the work. The writer wishes to thank Professor Babcock for his interest and guidance throughout the course of the work, and also to thank Professor R. E. Clausen and Dr. G. L. Stebbins, Jr., for their many helpful suggestions.

Acknowledgment is made of partial support of these investigations by grants from the Carnegie Institution of Washington and the Rockefeller Foundation; also to the Works Progress Administration for the services of a typist.

## MATERIALS AND METHODS

The cultures used in the investigation were:

(1) One collection of *C. divaricata* from the eastern promontory of Madeira, Promontory of San Lorenzo, Ilha de Cevada.

(2) One collection of *C. Noronhaea* from seed collected in Porto Santo and grown for one generation in the museum garden at Funchal.

(3) One collection of *C. canariensis* from Lanzarote Island in the Canary group.

(4) One collection of *andryaloides* from the mouth of the Ribeira do Inferno on the north coast of Madeira; the plants or the seeds had apparently been washed or blown down from the highlands.

(5) Three cultures of *taraxacifolia* collected in the vicinity of Funchal, on the south side of Madeira.

Collections of (1), (4), and (5) were made by Babcock in 1930, and the other two were sent to him at Berkeley in 1931.

Crosses were made between all five entities in May and June, 1933, and repeated in the following year; there was no obvious difference between the results in the two years. The method used was a slight modification of that described by Collins (1922).

All root tips were fixed in Muntzing's (1933) modification of Nava-shin's fixative, section at  $9\mu$ , and stained either with Haidenhain's iron-alum haematoxylin or Smith's (1934) modification of crystal violet. All meiotic figures were studied in acetocarmine, McClintock's (1929) technique being used. Pollen grains were mounted on a slide in a drop of acid fuchsin dissolved in lactic phenol, a medium which obviated the necessity of sealing the mounts. All the pollen counts were made after the plants had been in bloom for about two weeks.

## MORPHOLOGY OF THE PARENT SPECIES

It is not to be expected that seeds collected from a few plants in the wild would give plants showing the total variability of any one species. However, a comparison with specimens collected in the field showed that the cultures were a representative sample of the total variability of the species. The measurements can be taken as a fair approximation of what is characteristic of these species, and are adequate to establish the relative differences between the species.

The species were quite variable in themselves, as would be expected in self-incompatible ("self-sterile") plants. *Canariensis* and *divaricata* were more uniform than the other two, and it is interesting to note that

the first two species are the most restricted in their range, and may be considered as relic species.

In classifying the plants, there never was the slightest doubt of the species or subspecies to which they belonged. Each fluctuated about a distinct center of variability, and although there was frequently some overlapping in particular characters, yet in the sum total of characters the five entities were quite sharply delimited. The general appearance of all five is illustrated in plate 16, figures 7 to 12.

The differences between the species were expressed in all parts of the plants, principally in small differences of size and shape. Some of the more distinct differences are summarized in table 1, for purposes of illustration. In addition to quantitative character differences, there were a number of discontinuous variations peculiar to each of the species or subspecies, as, for example, a purple tip on the ligules of *canariensis*. All these latter characters were of no obvious adaptive significance; that is, they could be classified as nonessential. It is clear that the species can only be distinguished by means of a combination of characters. It was not possible, on the basis of external morphology of the plants grown in cultures, to divide the species into groups of a higher category.

### HYBRIDIZATION

Dobzhansky (1937, p. 231) has used the expression *incongruity of the parental forms* for "mechanisms [including geographic and ecological isolation] which prevent the production of hybrid zygotes, or engender such disturbances in the development that no hybrids reach the reproductive stage." Conversely, we may define the *congruity* (or genetic "compatibility") of two forms as their ability to hybridize and the  $F_1$  hybrids to form viable gametes (that is, gametes capable of producing vigorous zygotes). There is no simple way of measuring the congruity or expressing it by means of a single numerical value. Two forms may be so incongruous that they fail to produce any hybrid seed, owing to their incompatible reaction systems; or, on the other hand, they may be fully congruous, as is frequently true of varietal hybrids. Between these two extremes the incongruity may show up at various stages: the  $F_1$  zygotes may be so weak that they fail to germinate, or if they germinate they may die before maturity; the  $F_1$  plants may be quite as vigorous as the parental forms but closely approach complete sterility, for example, *Nicotiana sylvestris*  $\times$  *N. tomentosa*, Clausen (1928), *Primula verticillata*  $\times$  *floribunda*, Newton and Pellew (1929); finally, there are all degrees of fertility of the  $F_1$  hybrids, and all degrees of vigor of the  $F_2$  zygotes.

Consequently, an estimation of the percentage of viable gametes, in practice, entails: (1) a knowledge of the percentage of seed-setting on

TABLE 1

A COMPARISON OF THE PRINCIPAL MORPHOLOGICAL CHARACTERS IN *OREPIS CANARIENSIS*, *C. NORONHARA*, *C. DIVARICATA*, *C. VESICIGARIA* SUBSP. ANDREYALOIDES, AND *C. VESICIGARIA* SUBSP. TARAXACIFOLIA

| Character           | <i>canariensis</i>                   | <i>Noronhae</i>   | <i>divaricata</i>   | <i>andreyaloides</i>  | <i>taraxacifolia</i>  |
|---------------------|--------------------------------------|---|---|---|---|
| ROSETTE LEAVES      |                                      |   |   |   |   |
| Fine petiole        | Never                                | Usually   | Never   | Never   | Usually   |
| Winged petiole      | Always                               | Sometimes   | Always  | Always  | Rarely  |
| Length (mdn.)       | 13.0 cm*                             | 15.0  | 15.5  | 12.0  | 16.5  |
| (range)             | 6.0-18.0                             | 6.5-20.0  | 9.5-25.0  | 5.0-21.0  | 11.0-25.0   |
| Length/width (mdn.) | 3.2                                  | 5.0   | 3.8   | 4.2   | 4.6   |
| (range)             | 2.3-4.8                              | 3.2-8.7   | 2.9-4.8   | 2.9-8.0   | 3.2-6.0   |
| Dissection          | Denticulate or dentate               | Usually pinnately parted, rarely dentate  | Pinnately cleft or lobed, or dentate  | Pinnately parted  | Pinnately parted or divided, rarely dentate, frequently lobed       |
| Pubescence          | None                                 | Usually purplish setae, frequently none   | Usually purplish setae, frequently none   | Purple setae, and ciliated margin                             | Purple setae, sometimes ciliated margin; frequently neither         |
| Anthocyanin         | Frequently small dark definite spots | Sometimes small definite spots, sometimes larger lighter spots, sometimes red midrib; frequently no anthocyanin | Usually red midrib, sometimes large light spots, sometimes small dark spots; sometimes no anthocyanin | Usually large light spots, usually red midrib; rarely neither | Frequently large light spots, usually red midrib; sometimes neither |

| CAULINE LEAVES                           |   |   |  |  |  |  |
|--|---|---|--|--|--|--|
| Shape of the middle cauline leaves       | Cordate with clasping base                  | Usually linear or lanceolate, rarely oblanceolate | Usually cordate, sometimes lanceolate, or oblanceolate; usually with clasping base | Usually cordate, frequently oblanceolate; usually with clasping base | Usually linear or lanceolate; rarely with clasping base      |  |
| STEM                                     |   |   |  |  |  |  |
| Height (mdn.)                            | 30 cm.                                      | 28  | 46   | 27   | 28   |  |
| (range)                                  | 27-37                                       | 18-35   | 35-80  | 20-77  | 17-60  |  |
| Spread (mdn.)                            | 30 cm.                                      | 25  | 25   | 24   | 30   |  |
| (range)                                  | 25-40                                       | 13-43   | 13-60  | 12-50  | 15-53  |  |
| Height/spread (mdn.)                     | 1.0   | 1.0   | 1.6  | 1.5  | 0.9  |  |
| (range)                                  | 0.8-1.3                                     | 0.5-1.7   | 0.7-3.4  | 0.9-3.6  | 0.5-2.4  |  |
| Branching                                | Usually branched at base with basal foliage | Usually branched at base with basal foliage       | Usually regularly branched upwards with foliage evenly up the stem                 | Usually regularly branched upwards with foliage evenly up the stem   | Usually divaricately branched at the base with basal foliage |  |
| INFLORESCENCE                            |   |   |  |  |  |  |
| Number of heads on the ultimate branches | Sometimes 1-3, sometimes 4-5                | Usually 1-3, rarely 4-5                           | Sometimes 1-3, usually 4-5   | Usually 1-3, rarely 4-5  | Usually 1-3, rarely 4-5                                      |  |
| Pubescence of involucres and peduncles   | Always glandular hairs                      | Always glandular hairs, frequently blackish setae | Always glandular hairs   | Always glandular hairs, usually blackish setae                       | Usually glandular hairs, rarely absent                       |  |

\* All measurements are in centimeters or millimeters and are indicated by the first item in column one for each character.

TABLE 1—(Concluded)

| Character                         | <i>canariensis</i>              | <i>Noronhae</i>   | <i>dissecta</i>                              | <i>andryaloidea</i>                               | <i>larazae</i> /folia                             |
|-----------------------------------|---------------------------------|---|--|---|---|
| INFLORESCENCE—(Cont.)             |                                 |   |  |   |   |
| Mature bud (just before anthesis) |                                 |   |  |   |   |
| Length (mdn.) (range)             | 8.0 mm.<br>5.0-8.0              | 9.0<br>7.0-11.0   | 11.0<br>7.0-12.0                             | 8.0<br>4.0-9.0                                    | 9.0<br>5.0-10.0                                   |
| Bud                               |                                 |   |  |   |   |
| Length/width (mdn.) (range)       | 1.1<br>1.0-1.6                  | 1.6<br>1.4-1.8  | 1.6<br>1.4-2.2                               | 1.6<br>1.0-2.0                                    | 1.6<br>1.2-2.0                                    |
| Days from planting to flowering   | 141<br>109-352                  | 148<br>109-181  | 132<br>113-181                               | 419<br>120-473                                    | 155<br>122-214                                    |
| FLOWER                            |                                 |   |  |   |   |
| Diameter (mdn.) (range)           | 2.5 cm.<br>2.5-3.0              | 2.9<br>1.5-3.6  | 3.7<br>2.5-4.8                               | 2.6<br>2.3-2.8                                    | 2.8<br>2.0-3.3                                    |
| Ligule                            |                                 |   |  |   |   |
| Length (mdn.) (range)             | 12.0 mm.<br>12.0-15.0           | 14.0<br>11.0-16.0   | 18.0<br>16.0-20.0                            | 14.0<br>10.0-16.0                                 | 12.5<br>10.0-14.0                                 |
| Color                             | Lemon chrome† with a purple tip | Usually lemon yellow, sometimes lemon chrome; usually a red stripe on dorsal side | Usually lemon yellow, sometimes lemon chrome | Light lemon yellow, bleaching white when withered | Lemon yellow, usually a red stripe on dorsal side |
| Style-branches                    |                                 |   |  |   |   |
| Length (mdn.) (range)             | 2.3 mm.<br>2.2-2.4              | 2.2<br>1.8-2.5  | 2.5<br>2.0-3.0                               | 2.0<br>1.5-2.2                                    | 2.0<br>1.6-2.2                                    |

| Color   | Yellow  | Yellow   | Slightly greenish | Pale olive-green                                     | Pale olive-green |
|---|---|--|-------------------|--|------------------|
| Anther-tube<br>Length (mdn.)<br>(range)       | 3.2 mm.<br>3.0-3.5                                | 3.6<br>3.2-4.0                                       | 4.8<br>4.0-5.0    | 3.5<br>3.0-4.0                                       | 3.0<br>2.2-3.5   |
| Anther-appendages<br>Length (mdn.)<br>(range) | 0.6 mm.<br>0.5-0.7                                | 0.6<br>0.5-0.6                                       | 0.7<br>0.6-0.8    | 0.5<br>0.4-0.5                                       | 0.4<br>0.3-0.5   |
| INVOLUCRE                                     |   |  |                   |  |                  |
| Outer bracts, number<br>(mdn.)<br>(range)     | 10<br>10  | 5<br>5-8   | 9<br>7-12         | 6<br>4-8   | 7<br>6-8         |
| Inner bracts, length<br>(mdn.)<br>(range)     | 10.0 mm.<br>10.0                                  | 11.0<br>9.0-12.0                                     | 12.0<br>12.0      | 8.0<br>8.0-9.0                                       | 9.5<br>9.0-10    |
| ACHENES                                       |   |  |                   |  |                  |
| Color   | Usually cinnamon<br>brown, rarely<br>Rood's brown | Sometimes Vandyke<br>brown, sometimes<br>clove brown | Vandyke brown     | Frequently Rood's<br>brown, usually<br>Vandyke brown | Cinnamon brown   |
| Length (mdn.)<br>(range)                      | 5.2 mm.<br>4.0-6.0                                | 5.8<br>4.0-8.0                                       | 6.5<br>5.6-8.7    | 5.5<br>5.0-6.5                                       | 7.8<br>6.4-10.0  |
| Beak length (mdn.)<br>(range)                 | 1.4 mm.<br>0.9-2.2                                | 1.5<br>1.0-3.0                                       | 1.6<br>1.0-2.5    | 1.8<br>1.0-3.0                                       | 3.0<br>2.4-5.0   |
| Pappus length (mdn.)<br>(range)               | 3.6 mm.<br>2.7-5.0                                | 4.0<br>3.0-4.5                                       | 4.5<br>3.5-5.2    | 4.0<br>3.5-4.7                                       | 4.9<br>3.5-6.0   |

† Ridgway's Color Standards and Nomenclature used throughout.



the  $F_1$  plants, and (2) the raising of an  $F_2$  population in order to determine whether or not the gametes are capable of producing vigorous zygotes. For a complete conception of the congruity there are also necessary: (3) an estimate of the percentage of hybrid seed obtained from crossing the species, (4) a record of the germination of that seed, and (5) a knowledge of the percentage of  $F_1$  plants that grew to maturity.

The data on congruity for the ten possible hybrids are given in table 2. It might be well to discuss at some length each of the measures.

TABLE 2

PERCENTAGE OF HYBRID SEED-SETTING, PERCENTAGE OF GERMINATION OF THE RESULTING SEEDS, PERCENTAGE OF MORPHOLOGICALLY GOOD POLLEN ON THE  $F_1$  PLANTS, PERCENTAGE OF OPEN FERTILITY OF THE  $F_1$  PLANTS, AND GERMINATION OF THE  $F_2$  SEEDS, OF THE TEN POSSIBLE HYBRIDS

| Cross   | Hybrid seed-setting | Germination of hybrid seed | "Good" pollen on $F_1$ | $F_1$ Fertility | Germination of $F_2$ seed |
|---------|---------------------|----------------------------|------------------------|-----------------|---------------------------|
|         | 1                   | 2                          | 3                      | 4               | 5                         |
| T-A*    | 54 (1)†             | 71 (5)                     | 81 (1)                 | Fair‡ (3)       | 35                        |
| N-A*    | 44 (2)              | 86 (1)                     | 46 (5)                 | Fair (4)        | 88                        |
| N-T     | 42 (3)              | 76 (3)                     | 44 (6)                 | Poor (8)        | 20                        |
| D-A     | 42 (4)              | 62 (7)                     | 10 (10)                | Good (1)        | 47                        |
| D-N     | 38 (5)              | 43 (10)                    | 19 (9)                 | Very poor (10)  | 54                        |
| D-T     | 36 (6)              | 68 (6)                     | 58 (2)                 | Good (2)        | 67                        |
| D-C     | 36 (7)              | 48 (9)                     | 56 (3)                 | Fair (5)        | 43                        |
| A-C     | 24 (8)              | 75 (4)                     | 33 (8)                 | Fair (6)        | 50                        |
| N-C     | 16 (9)              | 53 (8)                     | 35 (7)                 | Poor (9)        | ..                        |
| T-C     | 10 (10)             | 86 (2)                     | 53 (4)                 | Fair (7)        | ..                        |
| Average |                     |                            |                        |                 |                           |
|         | 34                  | 67                         | 44                     | Fair            | 44                        |

\* T, *vesicaria* subsp. *tarazacifolia*; A, *vesicaria* subsp. *andryaloides*; N, *Noronhaea*; D, *divaricata*; C, *canariensis*.

T-A includes both combinations, namely, *tarazacifolia* ♀ × *andryaloides* ♂ and *andryaloides* ♀ × *tarazacifolia* ♂. The other nine combinations also include the reciprocals.

† The numbers in parentheses refer to the relative order of the observation in magnitude array, beginning with the highest.

‡ Excellent, 76-100 per cent; good, 51-75; fair, 26-50; poor, 3-25; very poor, 1-2.

The percentage of hybrid seed-setting (table 2, column 1) is merely the ratio, expressed in percentage, of seeds obtained to the number of florets emasculated. Approximately one hundred florets were emasculated in each cross, with conditions kept as nearly constant as possible. No difference was noted in reciprocal crosses. It may be significant that when the percentages are arranged in magnitude array, crosses between *tarazacifolia* and *andryaloides* stand at the top; these two subspecies are hybridizing in nature and are producing many intermediate progeny. There is practically no difference between the various *divaricata* and *Noronhaea* crosses, and finally, the crosses involving *canariensis* are all

at the bottom of the list. The numbers and the samplings of the native populations of the species are not sufficient to establish any precise degree of relationship on this basis.

The percentage of germination (table 2, cols. 2 and 5) of the  $F_1$  and  $F_2$  seed was very little different from that of the parents; the average germination for the four species, including the two subspecies of *vesicaria*, was 45 per cent—varying from 25 per cent for *canariensis* up to 86 per cent for *andryaloides*. There was no obvious difference in the reciprocal crosses, and the average for the various crosses did not differ markedly from the average for all the crosses. Practically all the  $F_1$  seeds that germinated grew into plants which lived to maturity; the few that died in the course of the experiment did so from causes far removed from inharmonious combinations of genes.

**Fertility** (table 2, col. 4).—A study of the fertility of the hybrids is complicated by the situation in the pure species, where, with the exception of subsp. *taraxacifolia*, the parents are completely or almost completely self-incompatible. However, an abundance of seed was obtained when the heads of sister plants were rubbed; also, the fertility of the open-pollinated plants was high, particularly when exposed to the visitations of insects.

An attempt was made with *canariensis* to see whether there were definite intrasterile-interfertile groups within the species, as East (1932) and others obtained in *Nicotiana* and other genera. The results did not conform to a simple scheme. If there was a single series of fertility allelomorphs, their clear-cut expression was modified by other factors, either modifying genes or fluctuations of the environment.

It was difficult to measure the degree of fertility accurately; so the percentage of seed-setting was estimated as belonging to four groups: excellent (76–100), good (51–75), fair (26–50), and poor (1–25); the last class was subdivided into a classification of very poor (1–2).

The open seed-setting on the  $F_1$  hybrids was markedly poorer than that of the parents, where the open seed-setting was usually 100 per cent, or at least in the excellent class. Also there was a decrease in the average amount of morphologically good pollen (table 2, col. 3); although, even in the parents, which usually had from 80 to 100 per cent of apparently good pollen, sometimes there was as low as 20 per cent, in spite of the fact that the flowers were selected when the plants were at the height of their blooming season (that is, after they had been in flower for about two weeks). As a consequence of this variability, the amount of good pollen could not be used as an index of fertility. If there was some simple relationship existing between the amount of apparently good pollen and the seed-setting, it would have been very difficult to establish without an extensive statistical study.

Under the most favorable conditions of seed-setting, open-pollinated heads sometimes appeared on some  $F_1$  plants in which all the possible embryo sacs had developed. Consequently, the normal procedure in gonogenesis being assumed, there is every indication that under certain circumstances all the female gametes in these particular hybrid plants are capable of functioning.

*Conclusions from hybridization.*—(1) All five entities hybridize very readily. (2) The crossability of the various species and subspecies, taken in pairs, was about the same, though there was a suggestion that *canariensis* was less congruous ("compatible") than the others. (3) The hybrid seeds germinated as well as those of the parents, with little or no difference between the individual crosses. (4) The hybrids, on the whole, were less fertile than the parents, and there was less morphologically good pollen, but the correlation was not obvious. (5) Hybrids involving *taraxacifolia* were slightly more fertile than the others. (6) Under certain circumstances, all the gametes in some hybrids were capable of functioning.

### CYTOLOGY

All five species and subspecies had eight chromosomes at the mitotic metaphase, confirming the observations of Babcock and Cameron (1934). Although several plates from each were carefully measured, there



Fig. 2. Somatic metaphase from root-tip cells of: a, *Crepis vesicaria* subsp. *taraxacifolia*; b, *Crepis canariensis*  $\times$  *C. vesicaria* subsp. *andryaloides*  $F_1$ .  $\times 2500$ .

All parts of this figure were drawn with the aid of a camera lucida at a magnification of 3750 times, from permanent preparations, and reduced one-third in reproduction.

seemed to be no constant difference either in morphology or in total length of the various chromosome types. The differences observed between them were small and could easily be explained on the basis of variations in fixation, age of the cells, twists, etc. The somatic chromosomes of *taraxacifolia* are illustrated in text figure 2, a, which would serve equally well for any of the parents (see also Babcock and Cameron, 1934).

In the first-generation hybrids the somatic metaphase chromosomes appeared to be the same as in the parents. In size, staining capacity, and morphology the maternal and paternal elements could not be distinguished. Both of the D chromosomes in the hybrids had a satellite, that is, there was no indication of amphiplasty as reported by Navashin (1928) and Hollingshead (1930) in more distant species hybrids in *Crepis*. Text figure 2, *b* shows a somatic plate of the  $F_1$  hybrid *canariensis*  $\times$  *andryaloides*, which is essentially similar to the parents.

**Meiotic chromosomes.**—Both in the hybrids and in the parents, at first meiotic metaphase four bivalent chromosomes were regularly seen, all of which disjoined in a normal fashion (see text fig. 3, *b* and *c*). Also, the second meiotic division was normal, and comparable, in all respects, in the parents and in the hybrids.

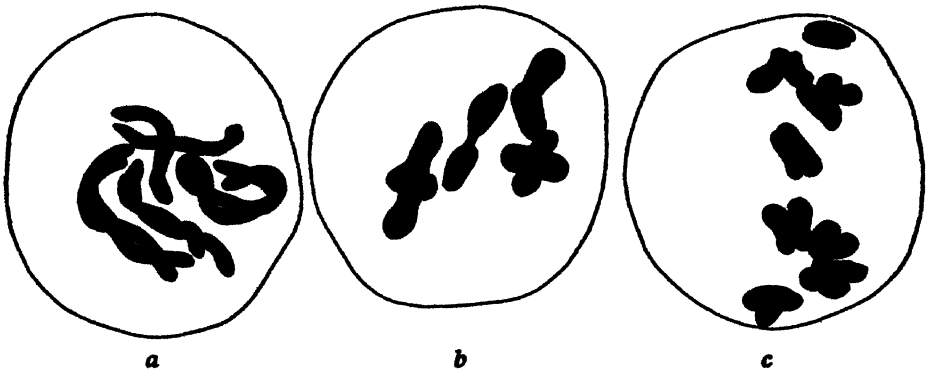


Fig. 3. Meiosis in *Crepis Noronhaea*  $\times$  *C. canariensis*  $F_1$ : *a*, diplotene showing four paired elements; *b*, metaphase, showing four typical bivalent chromosomes; *c*, anaphase, showing four chromosomes passing to each pole.  $\times 1700$ .

All parts of this figure were drawn with the aid of a camera lucida at a magnification of 3400 times, from acetocarmine preparations which were squashed by light pressure, and reduced one-half in reproduction.

The parents frequently have a single nonterminal chiasma at early diakinesis (see text fig. 3, *a*), the minimum required to maintain pairing. Earlier stages were not examined in detail in regard to chiasma frequency, as it is almost impossible to distinguish between twists and chiasmata in acetocarmine preparations. At late diakinesis there was usually one, sometimes two chiasmata, and rarely three. This is rather surprising in view of the great length of the *Crepis* chromosomes. Another curious fact is that there was little terminalization until late diakinesis or early metaphase.

Recently, Darlington (1931) has regarded the relative frequency of chiasmata formed in the first meiotic division of the parents and the hybrids between them as a measure of the genetic homology of the chromosomes. The work of McClintock (1933) on nonhomologous association and Beadle (1933) on asynaptic maize, and of Kihara (1929) and others

on the influence of temperature, would throw considerable doubt on this measure of relationship. Nevertheless, if this criterion has any value whatever, these species are very closely related.

The cytological evidence strongly indicates that the five entities have a similar arrangement of genes in the various chromosome types. In other words, there have been no large duplications, translocations, or other rearrangements that in any way interfere with normal meiosis.

### HYBRID SEGREGATION

The five entities were crossed in all possible ways, making ten different hybrid combinations, each including the reciprocal hybrid.  $F_2$  cultures were grown from all except two combinations, namely, *taraxacifolia-canariensis* and *Noronhaea-canariensis*, in which no selfed or sibbed seed was obtained. The behavior in all the hybrids was remarkably similar and of a type frequent in crosses between closely related forms. For the sake of brevity, the general behavior of the hybrids will be described and illustrated by data from only one hybrid combination, namely, *taraxacifolia-divaricata*.

The  $F_1$ -generation plants were variable, but no more so than the parents. The character differences were manifested in either of two ways: (1) they were more or less intermediate between the parental averages, or (2) the influence of one parent was more pronounced. The greater number of the characters were of the intermediate type, which included practically all the distinctive species differences; for example, height, flower size, achene size and color, and beak length. Each hybrid was distinctive, and it was easy to determine the parental species by an inspection of the hybrid; thus no species was entirely dominant over any other species. The characters that showed dominance were, for the most part, those which had no apparent adaptive significance; for example, anthocyanin patterns, pubescence, color patterns (see pl. 16, figs. 1-6 for the appearance of the rosettes of another series of hybrids).

The  $F_1$ -generation plants were just as vigorous as the parents, but no more so. This lack of hybrid vigor is probably to be explained by the consistent cross-pollination of the wild species, which makes them highly heterozygous.

In the  $F_2$ , most of the characters followed the blending type of inheritance, even most of those in category 2 above. This shows that the specific and subspecific complexes were made up of a large number of genes, and that most of the characters, if not all of them, were influenced directly by many genes. A few characters, those determined by a single gene differential, showed dominance in the  $F_2$ .

The characteristics of the blending inheritance in these species hybrids, as illustrated in tables 3-9, may be roughly summarized as follows:

(1) In both  $F_1$  and  $F_2$ , there was a continuous range of expression of any one character difference, with the majority of the individuals intermediate. The mean of the  $F_2$  population was similar to that of the  $F_1$ . (2) The range of the  $F_2$  variation was about that of the parental extremes, with no well-marked occurrences of transgressive inheritance. This lack may have been due to the small numbers, as the  $F_2$  population in any one cross did not exceed one hundred individuals; on the other hand, it may have been due to the lack of dominance in the various gene series. (3) There was no recovery of types corresponding to the parents in all or most of the characters. In many hybrids, particularly where there are chromosomal difficulties, the intermediate types are eliminated, that is, they are unfavorable combinations. The fact that no parental types were recovered indicates (a) that the intermediate combinations, or at least a large number of them, were able to survive, and (b) that there were a great many genic differences between the two parents. (4) No new characters appeared in either  $F_1$  or  $F_2$ , which would indicate that the gene systems in all the species and subspecies were essentially the same. The new combinations in the hybrids merely altered the expression of the existing characters.

It is now known that multiple genes can bring about such a continuously varying  $F_2$ , that is, several genes, each with a small increment, affecting the same character. It is very probable that species that are highly heterozygous will have a blending type of inheritance, or at least a variable expression, for most if not all characters. It is only in relatively homozygous lines that it would be possible to obtain a sufficient number of clear-cut segregations to reveal the genetic basis of such small character differences as those found in these species. It would be a long and tedious piece of work to put these interspecific differences on a Mendelian basis, and the task would be greatly complicated by the presence of self-incompatibility.

Data were taken on a number of more or less clear-cut character differences between the species and subspecies. With the small number of individuals, and in the limited time, it was not possible to work out the factorial bases of these characters beyond saying that they are gene determined but do not conform to a simple Mendelian scheme. The results in one are more or less typical of them all, and for the sake of brevity only one will be illustrated.

Both *taraxacifolia* and *Noronhaea* have a conspicuous red stripe on the dorsal surface of the outer row of ligules, which was somewhat variable in its expression. The other three, namely, *canariensis*, *divaricata*, and *andryaloides*, had no stripe, and it did not appear in any of the six possible hybrids between them.

In all the crosses in which one parent had a stripe and the other had

TABLE 3

FREQUENCY DISTRIBUTIONS OF ROSETTE LEAF LENGTH IN *C. DIVARICATA* AND *C. VESICARIA* SUBSP. *TARAXACIFOLIA*, AND THE FIRST- AND SECOND-GENERATION HYBRIDS BETWEEN THEM

| Species and hybrids         | 9* | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | Total |
|-----------------------------|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|-------|
| <i>Divaricata</i> .....     | 1  | 3  | 5  | 3  | 3  | 2  | 3  | 3  | 5  | 3  | 2  | 4  | 1  | 1  |    |    | 1  |    | 40    |
| <i>Taraxacifolia</i> .....  |    |    | 1  | 3  | 7  | 7  | 7  | 5  | 1  | 8  | 5  | 4  | 4  | 3  | 2  | 2  | 1  |    | 60    |
| <i>F</i> <sub>1</sub> ..... |    | 5  | 5  | 4  | 6  | 4  | 3  | 2  | 2  | 1  |    |    |    |    |    |    |    |    | 32    |
| <i>F</i> <sub>2</sub> ..... | 3  | 4  | 10 | 5  | 8  | 12 | 16 | 14 | 12 | 15 | 7  | 6  | 8  | 2  | 2  |    | 2  | 1  | 127   |

\* Only the lower limits of the class intervals are stated, in centimeters.

TABLE 4

FREQUENCY DISTRIBUTIONS OF THE RATIO OF THE LENGTH TO THE WIDTH OF ROSETTE LEAVES IN *C. DIVARICATA* AND *C. VESICARIA* SUBSP. *TARAXACIFOLIA*, AND THE FIRST- AND SECOND-GENERATION HYBRIDS BETWEEN THEM

| Species and hybrids         | 2.8 | 3.0 | 3.2 | 3.4 | 3.6 | 3.8 | 4.0 | 4.2 | 4.4 | 4.6 | 4.8 | 5.0 | 5.2 | 5.4 | 5.6 | 5.8 | 6.0 | 6.2 | 6.4 | Total |
|-----------------------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-------|
| <i>Divaricata</i> .....     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | 40    |
| <i>Taraxacifolia</i> .....  | 1   | 4   | 6   | 5   | 5   | 4   | 9   | 2   | 3   | 1   | 7   | 5   | 5   | 2   | 1   | 2   | 2   |     |     | 60    |
| <i>F</i> <sub>1</sub> ..... |     |     | 1   | 3   | 1   | 5   | 5   | 4   | 4   | 2   | 2   |     | 4   |     | 1   |     |     |     |     | 32    |
| <i>F</i> <sub>2</sub> ..... | 1   | 4   | 5   | 5   | 9   | 12  | 9   | 12  | 14  | 13  | 9   | 13  | 9   | 3   | 2   | 5   | 1   | 1   |     | 127   |

\* Only the lower limits of the class intervals are stated, in centimeters.

TABLE 5

FREQUENCY DISTRIBUTIONS OF THE DIAMETER OF THE FLOWER IN *C. DIVARICATA* AND *C. VESICARIA* SUBSP. *TARAXACIFOLIA*, AND THE FIRST- AND SECOND-GENERATION HYBRIDS BETWEEN THEM

| Species and hybrids         | 1.8* | 2.0 | 2.2 | 2.4 | 2.6 | 2.8 | 3.0 | 3.2 | 3.4 | 3.6 | 3.8 | 4.0 | 4.2 | 4.4 | 4.6 | 4.8 | Total |
|-----------------------------|------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-------|
| <i>Divaricata</i> .....     |      |     |     | 1   |     | 2   | 2   | 2   | 4   | 2   | 2   | 2   | 4   | 3   | 1   | 1   | 26    |
| <i>Taraxacifolia</i> . .... |      | 3   | 3   | 5   | 5   | 4   | 10  | 2   |     |     |     |     |     |     |     |     | 32    |
| F <sub>1</sub> .....        |      |     | 2   | 1   |     | 3   | 6   | 8   | 5   | 3   |     | 2   |     |     |     |     | 30    |
| F <sub>2</sub> .....        | 1    | 4   | 2   | 14  | 7   | 7   | 24  | 22  | 7   | 1   | 2   | 2   |     |     |     |     | 93    |

\* Only the lower limits of the class intervals are stated, in centimeters.

TABLE 6

FREQUENCY DISTRIBUTIONS OF HEIGHT OF STEM IN *C. DIVARICATA* AND *C. VESICARIA* SUBSP. *TARAXACIFOLIA*, AND THE FIRST- AND SECOND-GENERATION HYBRIDS BETWEEN THEM

| Species and hybrids         | 15* | 20 | 25 | 30 | 35 | 40 | 45 | 50 | 55 | 60 | 65 | 70 | 75 | 80 | Total |
|-----------------------------|-----|----|----|----|----|----|----|----|----|----|----|----|----|----|-------|
| <i>Divaricata</i> .....     |     |    |    |    | 3  | 7  | 8  | 2  |    |    |    |    |    | 1  | 21    |
| <i>Taraxacifolia</i> . .... | 1   | 4  | 9  | 1  | 2  | 5  | 2  | 2  |    | 1  |    |    |    |    | 27    |
| F <sub>1</sub> .....        |     |    | 3  | 2  | 5  | 9  | 4  | 6  | 1  |    |    |    |    |    | 30    |
| F <sub>2</sub> . ....       | 1   | 8  | 6  | 9  | 11 | 15 | 9  | 18 | 5  | 10 | 3  | 4  | 3  | 1  | 103   |

\* Only the lower limits of the class intervals are stated, in centimeters.



TABLE 7

FREQUENCY DISTRIBUTIONS OF THE RATIO OF HEIGHT TO SPREAD IN *C. DIVARICATA* AND *C. VESICARIA* SUBSP. *TARAXACIFOLIA*, AND THE FIRST- AND SECOND-GENERATION HYBRIDS BETWEEN THEM

| Species and hybrids         | 0.4* | 0.6 | 0.8 | 1.0 | 1.2 | 1.4 | 1.6 | 1.8 | 2.0 | 2.2 | 2.4 | 2.6 | 2.8 | 3.0 | 3.2 | 3.4 | Total |
|-----------------------------|------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-------|
| <i>Divaricata</i> .....     |      | 2   | 3   | 3   | 1   | 1   | 1   |     | 1   | 2   | 2   | 2   |     | 1   |     | 1   | 20    |
| <i>Taraxacifolia</i> ..     | 6    | 7   | 1   | 2   |     | 1   | 3   | 2   | 3   |     | 2   |     |     |     |     |     | 27    |
| <i>F</i> <sub>1</sub> ..... |      | 3   | 4   | 3   | 8   | 3   | 2   | 2   | 2   | 1   | 1   | 1   |     |     |     |     | 30    |
| <i>F</i> <sub>2</sub> ..... | 19   | 10  | 14  | 14  | 5   | 8   | 5   | 6   | 5   | 7   | 3   | 4   | 2   | 1   |     |     | 103   |

\* Only the lower limits of the class intervals are stated, in centimeters.

TABLE 8

FREQUENCY DISTRIBUTIONS OF LENGTH OF ACHENES IN *C. DIVARICATA* AND *C. VESICARIA* SUBSP. *TARAXACIFOLIA*, AND THE FIRST- AND SECOND-GENERATION HYBRIDS BETWEEN THEM

| Species and hybrids         | 5.0* | 5.5 | 6.0 | 6.5 | 7.0 | 7.5 | 8.0 | 8.5 | 9.0 | 9.5 | 10.0 | Total |
|-----------------------------|------|-----|-----|-----|-----|-----|-----|-----|-----|-----|------|-------|
| <i>Divaricata</i> .....     |      | 2   | 7   | 6   | 5   | 1   |     | 1   |     |     |      | 22    |
| <i>Taraxacifolia</i> .....  |      |     | 1   | 5   | 5   | 5   | 9   | 2   | 2   |     | 1    | 30    |
| <i>F</i> <sub>1</sub> ..... |      |     | 1   | 5   | 3   | 6   | 3   | 1   |     |     |      | 19    |
| <i>F</i> <sub>2</sub> ..... | 4    | 3   | 4   | 17  | 14  | 29  | 14  | 8   | 4   |     |      | 97    |

\* Only the lower limits of the class intervals are stated, in millimeters.

TABLE 9  
FREQUENCY DISTRIBUTIONS OF LENGTH OF BEAK OF ACHENES IN *C. DIVARICATA* AND *C. VESICARIA* SUBSP. *TARAXACIFOLIA*, AND THE FIRST- AND SECOND-GENERATION HYBRIDS BETWEEN THEM

| Species and hybrids            | 1.0* | 1.5 | 2.0 | 2.5 | 3.0 | 3.5 | 4.0 | 4.5 | 5.0 | Total |
|--------------------------------|------|-----|-----|-----|-----|-----|-----|-----|-----|-------|
| <i>Divaricata</i> . . . . .    | 7    | 5   | 9   | 1   | 8   | 8   | 1   |     | 1   | 22    |
| <i>Taraxacifolia</i> . . . . . |      |     | 2   | 10  | 4   | 3   |     |     |     | 30    |
| F <sub>1</sub> . . . . .       |      | 2   | 4   | 6   | 28  | 13  | 1   | 1   |     | 19    |
| F <sub>2</sub> . . . . .       | 2    | 11  | 11  | 30  |     |     |     |     |     | 97    |

\* Only the lower limits of the class intervals are stated, in millimeters.

none, the  $F_1$  segregated 59 plants with a stripe to 14 without, and the  $F_2$  segregated 99 plants with a stripe to 43 without. In the  $F_2$ , the deviation from a 3:1 ratio is not quite significant, since a deviation as large as this would be expected in slightly more than one out of ten, from chance alone. Progeny were obtained from 5 of the 14 plants in  $F_1$  that did not have a stripe. Four of the 5 segregated  $F_2$  plants with a stripe, indicating that the  $F_1$  plants carried the red-stripe gene though it was not expressed. The fifth  $F_1$  plant did not have a stripe, nor did any of its 10 progeny. There is some evidence that the *taraxacifolia* plant used in the original cross was heterozygous, as 2 of its progeny out of a total of 9 were without a stripe. If this latter progeny is excluded from the total for the  $F_2$ , the ratio is 99:33, a perfect agreement with a 3:1 ratio.

It is most probable that the  $F_1$  parents of those  $F_2$  progenies that segregated red-striped plants although their  $F_1$  parents had none, did not have the proper genetic background for the gene to express itself, but that the  $F_2$  recombinations did supply the favorable background; that is, the presence of this character not only requires the presence of the gene in the dominant condition, but also requires a definite genic background, much as was observed of Harland's (1935) crinkled dwarf in *Gossypium*, which showed different expressions of the character, depending upon the particular genic milieu in which it had to develop. Further evidence for this theory is the wide range of expression of the character in  $F_1$ , where some plants had such a slight expression that they were difficult to distinguish from normal, and an even wider range in  $F_2$ , where some of the latter plants were so intense in their expression that the color showed through on the upper side of the ligule.

In crosses between *taraxacifolia* and *Noronhaea*, both of which had a stripe, all the  $F_1$  showed the stripe; and out of 14  $F_2$  plants 3 had no stripe and all these occurred in the progeny of the same  $F_1$  plant. This lack of the red stripe may be due (1) to a slight expression which was overlooked in the classification, or (2) to the wrong background for the visible expression of the gene.

In spite of the fact that the numbers are small and the evidence somewhat conflicting, it appears that the stripe may be referred to a single dominant gene which behaves in a normal Mendelian manner; though the expression of the character is dependent, to an appreciable degree, upon modifying genes. It is probable, for instance, that if the red-stripe gene were introduced into the *divaricata* background by repeated backcrosses, it would segregate in a normal Mendelian fashion but might not show the same dominance relationships or expression that it shows in *taraxacifolia*. There is probably enough difference in the genic background to suggest that the expression of this gene would be modified in the new background.

## DISCUSSION

These five entities can be readily distinguished from one another by observation; the morphological differences between them are expressed as many small differences in size, color, and shape, affecting almost all plant organs. There are a few outstanding qualitative differences between them, though these are, taxonomically, of a minor nature; for example, the purple tip on the ligules of *canariensis*, the ligules withering white in *andryaloides*.

Two of the species, *canariensis* on Lanzarote Island of the Canary group and *Noronhaea* on Porto Santo Island, are geographically well isolated from the other two on Madeira. Of the Madeiran species, *divaricata* and *vesicaria andryaloides* occupy different ecological stations, the former being found only on Promontory San Lorenzo, which is an island at high tide, and the latter in the northern highlands of Madeira. In spite of the fact that these species have occupied these regions for a long time, there is no evidence that they have ever hybridized in nature. *Vesicaria taraxacifolia*, on the other hand, is undoubtedly of more recent advent on the island, probably having been introduced by the early settlers around Funchal, on the south coast. It is well established there and has spread to the north side of the island, particularly around the vineyards; furthermore, it is an "aggressive" weedy type and is spreading. In the north-central part of Madeira, where *taraxacifolia* and *andryaloides* have come into contact, numerous intermediate forms were collected and observed by Babcock in 1930. These are undoubtedly natural hybrids. But there was no evidence that *taraxacifolia* had spread to the eastern end of the island and hybridized with *divaricata*.

The fact that all five entities have the same karyotype and that the chromosomes apparently mate up chromomere for chromomere in the meiotic prophase of the hybrid, with no subsequent irregularity, would indicate that they have essentially the same genic arrangement. It is difficult to prove that all five have the same number of genes, though the evidence points to this conclusion. There may be minute rearrangements and even lack of some particular genes in some of the species; however, if this is so, it is not reflected either in the pairing of the hybrids or in a constant elimination of large proportions of gametes.

The hybridization experiments demonstrate that the species and subspecies are able to exchange genes readily. The hybrids are produced without difficulty and show a fair measure of fertility; only a comparatively small proportion of the hybrid recombinations are incapable of surviving. The hybrid cultures gave every evidence of being as vigorous as the parental species, and were quite as vigorous as the progeny of some natural hybrid derivatives of *taraxacifolia* and *andryaloides*.

All the evidence is consistent with the view that the five entities have a great many gene differences, though the number must remain problematical. The most probable assumption is that all the species and subspecies possess the same number of genes, but that there are many different combinations of alleles. In any one species there must be a considerable proportion of heterozygous genes, and since the range of variability in the  $F_2$  for most characters is roughly twice that of the parents, there must be a higher proportion of heterozygous genes in the hybrids between the species and subspecies.

The prevalent type of  $F_2$  segregation for any single character can be satisfactorily explained on the basis of four or five genes with incomplete dominance. But it is not likely that even a probable estimate of the total number of genic differences could be obtained by multiplying the number of character differences by four or five, as we know that many genes, if not all, may influence several characters. It is quite possible that a very few genes influencing growth rates at slightly different periods of development could produce a large array of character combinations. It would require a very long and extensive breeding program to establish with certainty the number of genes influencing any one character difference.

If the total number of basic genes available to these species is designated as  $a, b, c, d, \dots n$ , and it is assumed that each gene may have several alleles, which may be designated as  $a^1, a^2, a^3, \dots a^k; b^1, b^2, b^3, \dots b^k$ , etc., the gene population of each species would contain all  $n$  genes, but many, if not a majority, would be represented by two or more alleles clustered around what Wright (1932) calls an "adaptive peak." Two different specific combinations coming together in a hybrid do not upset the gene balance, but many of their segregation products (recombinations) are not equally viable: some are so unbalanced as to produce lethal or very weak combinations; in other words, they fall in the "adaptive valleys."

The genes in one species may be transferred to another, and although not all the hybrid combinations are equally successful and many are eliminated, it is possible that new and still more harmonious combinations might be built up. Some offspring might even be adapted to new habitats and start an independent line which in time might become ecologically distinct.

All the evidence indicates that the isolating mechanisms that have been built up between these species are due to gene incompatibilities, which undoubtedly have arisen by mutation over a long period of time, and there is no indication whatever of any chromosomal rearrangements. This is rather surprising in view of the ease with which quite radical rearrangements were obtained by Navashin and Gerassimova (1936)

through the ageing of *Crepis* seed, which would seem to be a natural process.

In *Crepis* there are several groups of morphologically closely related species with a similar karyotype (Babcock and Cameron, 1934). It is natural to assume that some of the differences in chromosome morphology between the groups are due to chromosomal rearrangements. Müntzing (1934) found evidence of one inversion between *C. divaricata* and *C. dioscoridis*, the former from subgenus *Barkhausia* and the latter from subgenus *Eucrepis*. There have been two additional instances (unpublished): *C. oporinoides*  $\times$  *patula*, two distantly related species of the subgenus *Eucrepis*; and *C. canariensis*  $\times$  *oporinoides*, the former of subgenus *Barkhausia* and the latter of *Eucrepis*. In the latter two hybrids there were extensive translocations, but there were also very obvious differences in the karyotypes, which would lead one to suspect that there had been translocations.

It is also natural to assume that the species within any one group have essentially the same arrangement of genes, particularly in view of the fact that the differences in chromosome number and morphology between groups are quite striking. Besides the present group, only one other closely related group of species with a similar karyotype has been investigated. Cave (1936) studied four such species: *Crepis foetida*, *C. commutata*, *C. eritrieensis*, and *C. Thomsonii*, with essentially the same result, namely, that there was no evidence of rearrangements. Consequently, the assumption of a similar arrangement of genes is borne out in these two investigations. Whether or not it is true in the whole genus will have to be determined by further research. Nevertheless, these two instances materially strengthen the evidence for the assumption that similar chromosome morphology, of closely related species within this genus, indicates structural similarity; accordingly, karyotype studies are valuable in determining genetic relationships.

These species and their close relatives have had a very complex evolutionary history, involving repeated isolations and hybridizations; so that it is impossible, with the available evidence, to trace their phylogeny in any detail. Since there are few qualitative variations differentiating the five species and subspecies, and since almost all these variations are present in at least two of them, it is probable that the majority of the specific differences were present in the ancestral stock. Nevertheless, some character differences have undoubtedly arisen since the separations, for example, the purple tips of the ligules in *canariensis*, and it is quite likely that the quantitative differences have been emphasized in the passage of time. The uniformity of the environment on the islands would tend toward uniformity and less evolution of the species, once they became established in a favorable habitat; furthermore, the relatively small

numbers characteristic of most island species would also automatically tend toward still more uniformity (Wright, 1932).

Since these entities could not be arranged into groups of a higher category, they must have migrated to their present situations at different times, or must have come from forms which had already differentiated; either would involve separate migrations. This, with the fact that the nearest relatives of *canariensis*, *Noronhaea*, *divaricata*, and *andryaloides* are *C. Fontiana*, from northwest Africa, and *C. Bourgeauii*, from southwest Spain, would lend support to Cockerel's (1928) hypothesis that the indigenous flora of Madeira came from the northeast and, since these are oceanic islands, that the facilities for transportation have been available at all times.

It might not be out of place to speculate on the probable future of these species, granting that the forces working today continue to operate in the same way. Distinct geographic barriers prevent an interchange of genes between *canariensis* and *Noronhaea*, and their allied species. It is reasonable to assume that they will continue to differ progressively from each other and from the Madeiran group, and, if they are able to survive, will continue to pile up genetic differences which will decrease their congruity.

The situation in Madeira is somewhat different. *Andryaloides* and *taraxacifolia* are at the present time forming hybrids, backcrosses, and complicated segregates. This "hybrid swarm" seems to have many vigorous and robust plants. It is probable that *andryaloides*, being a more primitive relic, to judge from its perennial habit, restricted range, and strict ecological requirement, will in the end be "swamped" by the more aggressive *taraxacifolia*. It is worth noting, however, that this is a very slow and gradual process. Since it is highly probable that some of Lowe's peculiar forms were hybrid derivatives, the two had come into contact over a century ago, perhaps much earlier. Yet most of *taraxacifolia* (in Madeira) and presumably most of *andryaloides* (in the highlands) are still unaffected by the mingling of the two at certain points. But *taraxacifolia* is known to be a montane plant in other countries; hence, in all likelihood, it will gradually invade the highlands, and the mingling of the two will continue.

In any event, *andryaloides* will have contributed a number of new genes to the invader; and this will afford possibilities of segregating out new combinations of characters that are even better than the present *taraxacifolia* combinations, making this latter subspecies even more aggressive.

*Divaricata*, with its more restricted range and apparently more primitive characters (perennial habit, large flowers and leaves) and more homozygous expression, which is probably due to the more limited num-

bers in the species, will very probably die out because of overgrazing by goats, or it may contribute something to the *andryaloides-taraxacifolia* complex; so that ultimately there will be one polymorphic species with many ecological types. In other words, hybridization may produce a degradation as well as a multiplication of forms, and this makes the probable phylogenetic history of any species a complex one.

Those forms which are separated by both geographic and sterility barriers are, unquestionably, species in the Linnean sense. If the geographic barriers are present without the sterility barriers, it is a matter of opinion what will be the most useful and satisfactory way of treating the groups without doing too great violence to the convenient morphogeographical system, and at the same time incorporating as much of the genetical data as possible. Goodwin (1937) with a very similar situation in *Solidago* feels that on morphological grounds, and for the sake of convenience, the species should be kept distinct even though they do form fertile hybrids.

In a recent paper Clausen, Keck, and Hisey (1936) have proposed a scheme based on Turesson's (1929) genecological system. The ecospecies (Linnean species) are the smallest units which are kept apart by the aid of an inner genetical balance mechanism; that is, their hybrids are partly sterile. The ecotypes (subspecies) produce fertile hybrids and are kept apart through their geographical or ecological isolation. In other words, the main point is whether the forms have fertile or only partly fertile hybrids. The difficulty in this scheme is that, in practice, the fertility varies from 0 to 100 per cent, and somewhere along this range a more or less arbitrary point must be selected in order to divide the species from the subspecies.

In the present investigation three of these five entities maintain their morphological distinctness mainly through geographical and ecological isolation, and the other two, *andryaloides* and *taraxacifolia*, are becoming merged. It is clear that the final decision on the taxonomic treatment of such genetically close entities or systems must involve some arbitrary definitions. Since practical considerations must inevitably go along with every scientific approach to these problems, the fact of geographic or ecological isolation may properly serve as an adequate basis for the recognition of *divaricata*, *Noronhaea*, *canariensis*, and *vesicaria*. Their internal isolating mechanisms are as yet only imperfectly developed; that is, they are only on their way to becoming distinct species. However, the fact that they are to some degree incongruous and with continued isolation will probably become more so as time goes on, together with their morphological distinctness, would seem to be enough to establish them as distinct species.

Nevertheless, there is still the possibility that, should the territory of



any one be invaded by another, the two thus coming together would undoubtedly hybridize. It would remain for the botanist of that time to determine, through field studies and an investigation of the viability and fertility of the hybrid derivatives under natural conditions, the fate of the two species involved.

### SUMMARY

The cytogenetic relationships of four closely related species of *Crepis*, namely, *C. canariensis*, *C. divaricata*, *C. Noronhaea*, and *C. vesicaria* subsp. *andryaloides* and *taraxacifolia*, were investigated. The evidence presented was derived from (1) a detailed morphological study of the parents and the hybrids between them, (2) a comparison of the somatic and meiotic chromosome situation of the parents and of the hybrids, and (3) the inheritance of a number of characters in the first- and second-generation hybrids.

Among the five entities there were a great many morphological differences which affected all parts of the plant. In the hybrids by far the greater number of these differences appeared to be the result of the presence of a large number of multiple genes. All five had a similar karyotype and the chromosome behavior in the hybrids was similar in every respect to that in the parents. The internal isolating mechanism between them was found to be incomplete, although varying degrees of congruity between them were indicated by the comparative fertility of the hybrids. For practical taxonomic purposes, the fact of geographic or ecologic isolation warrants the recognition of *C. divaricata*, *C. Noronhaea*, *C. canariensis*, and *C. vesicaria*, as species; whereas *andryaloides* and *taraxacifolia* must be considered as subspecies of *vesicaria*, because they are hybridizing in nature and are losing their morphological distinctness.

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## **EXPLANATION OF PLATE**

## PLATE 16

### ROSETTE LEAVES

1. *Crepis canariensis*. Note the almost entire margins and the winged petioles.
2. *Crepis vesicaria* subsp. *taraxacifolia*  $\times$  *C. canariensis* F<sub>1</sub>. Note the slenderer petiole and the rounded apex characteristic of *taraxacifolia*.
3. *Crepis divaricata*  $\times$  *C. canariensis* F<sub>1</sub>. Note the dissection on the upper half of the leaves very frequently found in *divaricata*.
4. *Crepis Noronhaea*  $\times$  *C. canariensis* F<sub>1</sub>. Note the lyrate leaves and the slender petiole frequently found in *Noronhaea*.
5. *Crepis vesicaria* subsp. *andryaloides*  $\times$  *C. canariensis* F<sub>1</sub>. Note the somewhat modified pinnate dissection characteristic of *andryaloides*.
6. *Crepis Noronhaea*  $\times$  *C. divaricata*. Note the characteristic *divaricata*-like dissection as in 3.

1-6 are approximately  $\frac{1}{12}$  their natural size.

### MATURE PLANTS

7. *Crepis canariensis*.
8. *Crepis vesicaria* subsp. *andryaloides*.
9. *Crepis divaricata*.
10. *Crepis vesicaria* subsp. *taraxacifolia*, spreading form.
11. *Crepis vesicaria* subsp. *taraxacifolia*, erect form.
12. *Crepis Noronhaea*.

The plants 7-12 are growing in 6-inch pots.



1 *canariensis*



2 F<sub>1</sub> *taraxacifolia*  
*canariensis*



3 F<sub>1</sub> *divaricata* ×  
*canariensis*



4 F<sub>1</sub> *Noronhoa*  
*canariensis*



5 F<sub>1</sub> *andryaloides* ×  
*canariensis*



6 F<sub>1</sub> *Noronhoa*  
*divaricata*



7 *canariensis*



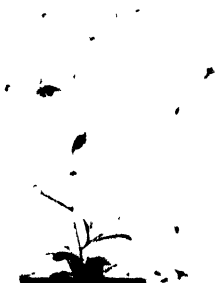
8 *andryaloides*



9 *divaricata*



10 *taraxacifolia*



11 *taraxacifolia*



12 *Noronhoa*



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